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RECENT ADVANCES IN PLANT PHYSIOLOGY

BY

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WITH A FOREWORD BY

PROFESSOR R. RUGGLES GATES

M.A., Ph.D.

*Professor of Botany in the University of London,
King's College*

WITH 51 ILLUSTRATIONS



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TO
MY FRIEND
AMES MONTAGU DRUMMOND, M.A.
REGIUS PROFESSOR OF BOTANY IN
THE UNIVERSITY OF GLASGOW

Printed in Great Britain

PREFACE

THE present text covers a strictly limited period ; approximately the years 1918 to 1928. An attempt has been made, however, to bring the subject-matter up to date, and some of the more relevant papers published during the present year (1929) have also been included.

Two choices were open to the author when he commenced this book : either to throw down before the reader a mass of names and references to the literature, or to discuss critically and in detail a few important and fundamental papers published in each year of the last decade. After a good deal of hesitation, the latter alternative was chosen. It will be seen that the bibliography is not extensive and makes no pretence to being exhaustive. The precise meaning that should be attached to the term " important " paper is largely one of personal taste, and the old adage " One man's meat is another man's poison " applies as well here as in other matters. The author has based his choice of papers entirely on his own personal predilections, and it is for others to say whether his choice be poor and indifferent, or if he has succeeded in steering a medium and reasonable course.

This book has been especially prepared for students, and not for specialists, and the needs of the former have been principally considered. There is at the present time no adequate text-book which covers the ground necessary for students reading for their final honours degree, and it is hoped that the present book fills the gap.

Stress has been laid on the economic aspects of the subject. Unless botanical laboratories are prepared to recognise the enormous economic possibilities of their subject, progress will be quickly stayed. The great advances plant physiology has made in America, with its separate societies and journals, is undoubtedly largely due to the fact that the economic, and not the purely

academic, side has been principally exploited. Agriculturists in this country have been slow to recognise the great importance to them of plant physiology. This state of affairs is not to be wondered at, and the blame must be laid at the door of plant physiologists. The plant physiologist, until comparatively recently, has been content to work on purely academic problems and has left the wider economic issues alone. Research for research's sake is an admirable slogan, but it has its limitations, and in the economic conditions at present prevailing in this country it is but natural that pure research with no economic outlook or possibilities is not held in such awe as in pre-war days. So little is the practical importance of plant physiology recognised here and in the Colonies that the author well remembers being told by a high Colonial agricultural officer: "Plant physiology; why, that is an amusing hobby!" Yet how many would care to stigmatise the work of Balls on the cotton plant in Egypt, or Denham and the work of the Shirley Institute in this country, as an "amusing hobby"? After all, the final explanation of the workings of the living organism must be given in terms of physics and chemistry, and that is the ultimate goal of physiology.

In the past plant physiology has occupied the "Cinderella" position in the botanical world. But the very active investigations that have taken place since the war have shown that many problems which appeared to be simple on the surface are in reality highly complex, and that plant physiology is not merely the contemplation of the obvious, as many have supposed. The whole situation, however, is now altered, and the very rapid strides that have been made in this branch of plant study have frequently brought forward the suggestion that it should be severed as a separate subject from botany. Such a separation would have many advantages, but it is probably outweighed by the disadvantages. It was an evil day for zoology when animal physiology was removed from it as a separate subject; and certainly botanical science would suffer from such a divorce.

There is a point which requires special emphasis here. Our present knowledge of plant metabolism is very seriously deficient in several directions. This unfortunate state of affairs is due to

several causes, among the principal of which is the fact that in plants several complex chemical reactions take place within the compass of a single cell, which makes the matter difficult of investigation. Nevertheless, the difficulty is intensified by the sporadic invasions of organic chemists into a domain of which they have little or no knowledge, with ready-made explanations based on *in vitro* experiments which are probably remote from the chemical reactions of the living plant. It is difficult to know why botanical physiology should be made the general playground for the imaginative theorising of persons who have no very reliable knowledge of the living plant.

The schools of plant physiology, founded by Professor V. H. Blackman at South Kensington and Dr. F. F. Blackman at Cambridge, have done an immense service in bringing the subject into prominence in this country. The work produced from their laboratories has always been of the highest type, and they and their co-workers, Dr. Knight and Dr. Gregory at South Kensington, and Dr. Briggs, Dr. Kidd and Dr. West at Cambridge, to mention but a few, have laid plant physiology under an immense burden of debt for their pioneer labours.

It is a pleasure to acknowledge the help I have received from numerous friends in the preparation of the text. I have to thank Professor R. Ruggles Gates for writing the Foreword and for his helpful criticism in many directions. To my wife and Mrs. V. E. Kastner I am indebted for reading the manuscript and for the preparation of some of the figures. I am very grateful to Professor V. H. Blackman, Professor Tansley, the Council of the Royal Society, Messrs. Longmans Green & Co., and a number of authors and publishers for allowing me to use illustrations from their published works. Mr. C. S. Semmens is responsible for the preparation of the majority of the figures, and I have to thank him for his untiring help. Lastly, to Messrs. J. & A. Churchill I owe a debt of gratitude for the very generous way in which they have always been ready to place their expert advice at my disposal.

FOREWORD

THIS volume is a summary of the more outstanding recent developments in plant physiology, and, as such, it should be of service not only to workers in this field, but also to other botanists and to those who are concerned with animal or human physiology. It is a fortunate circumstance that, although animal physiology is studied and taught as a science quite independent of zoology, plant physiology has always remained an integral part of botany. The correlated investigation of form and function is an ideal which is often expressed as one of the chief aims of biology, but owing to the divorce of physiology from zoology each of these sciences has gone its own way. It has been left for men styling themselves experimental biologists, and whose interests may be mainly botanical or mainly zoological, to take up anew the investigation of problems involving some knowledge and interest in the morphological as well as the physiological aspects of organisms. As regards plants, it has been a source of strength to botany that no such formal separation of its two main branches has ever taken place.

Although plant physiology has thus remained in intimate touch with the rest of botany, yet it has become in some respects an independent science, in relatively close relationship with chemistry, biochemistry and physics. Indeed, as will be seen from the contents of this book, the subject is ever reaching further in the application of data and methods from physics, chemistry and mathematics.

The six chapters of this book cover the main problems of plant physiology—namely, the soil and water relations of the plant, transpiration, photosynthesis, nitrogen metabolism, respiration, and growth. Each of these subjects has a long history, and only the more modern developments are considered, in such a way that the present status of the various questions can be seen. Inci-

dentially, recent work on related problems, such as the passage of stimuli in plants, the constitution of proteins and the function of respiratory pigments, is described. The work will thus be of much service to all those who wish to keep in touch with the latest developments.

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RECENT ADVANCES IN PLANT PHYSIOLOGY

CHAPTER I

THE SOIL AND WATER RELATIONS OF THE PLANT

Formation of the Soil—Soil Components and their Properties—Clay—Alkali Soils—Exchangeable Bases of the Soil—Flocculation—Humus—The Chemistry of Humus—Soil Moisture—Absorption of Water by the Root—Suction Pressure—The Soil Solution—Soil Acidity—Soil Reaction in Relation to Plant Growth and Distribution—Aeration—The Root System of Plants—Absorption of Salts—Antagonism—Effect of Temperature on Absorption of Salts—Mechanism of Entry of Salts into the Cell.

It is usually stated that it is only to the depth of some 8 to 9 inches that the soil is suitable for plant growth. This is the *soil* proper, and it gradually shades into the *subsoil* below, which in its turn passes by degrees into the underlying rock. The statement that the soil is the more important medium for plant nutrition and that the subsoil plays but an indirect part needs revision in the light of the recent investigation of Weaver, Jean and Crist (1922) on the root system of cereal crops. It now seems doubtful if such an assumption is valid (*cf.* section on Root System).

The true soil is formed by the process of weathering from rocks. Under such agencies as snow and frost, rain and air, the original rock is broken down and pulverised into smaller particles and gradually transformed into a suitable substratum for supporting plant life. Following on the primary break-up of the rock under the agencies described above, the solvent action of carbon dioxide is of considerable importance. A solution of this gas in water is weakly acid and gradually decomposes the felspars, double silicates of aluminium and either the alkali metals, sodium and potassium, or calcium, to aluminium silicate, which forms the

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main basis of clay. It possesses a more rapid action on limestone and chalk, which are principally composed of calcium carbonate.

A soil may be formed *in situ* or, as it is often termed, a *sedentary* soil, or, on the other hand, the eroded matter of the rocks may be carried away by rivers and streams and be eventually deposited in other parts of the globe as fresh beds of soil. Wind also helps in soil transport. Volcanic ash, for example, is carried considerable distances in this way.

The lower plants, lichens and blue-green algæ, play a part in the erosion of the rocks. They can exist on the bare rock surface and gradually penetrate the rock, thus making an entrance for the subsequent action of frost.

The first vegetation obviously obtains its mineral nutrients from the dissolved portions of the soil particles, but at death the decayed and decomposed plants return their mineral substance to the soil. Thus, later vegetation has a double source of supply of inorganic matter.

Should the dead vegetation lie on the surface of the soil, it suffers decay and may form peat. If, however, it becomes mingled with the soil—and this is more usually the case—it is transformed under the agency of bacteria and fungi to humus.

Soil Components and Their Properties

Agriculturists have been driven to composing a rough classification of the components of the soil based on the size of the particles.¹ This classification is purely one of convenience and very arbitrary in nature. It makes sharp divisions where only fine and indefinite gradations exist. The British classification is given below :—

| Diameter of Particles. | | | | |
|------------------------|---|---|---|----------------|
| Fine gravel | . | . | . | 3–1 mm. |
| Coarse sand | . | . | . | 1–0·2 mm. |
| Fine sand | . | . | . | 0·2–0·04 mm. |
| Silt | . | . | . | 0·04–0·01 mm. |
| Fine silt | . | . | . | 0·01–0·002 mm. |
| Clay | . | . | . | 0·002 and less |

¹ It is unfortunate in this connection that three different standards are now in use : British, Continental, and American.

Coarse sand plays an important part in keeping the soil open and helping to lighten heavy soils. Sand is non-colloidal in nature, and does not possess the power of adsorption. Soils with a high sand content are unfertile, and can only be properly cultivated after large additions of dung.

In nearly all soils the fine sand fraction forms a considerable proportion. Unlike coarse sand, it possesses a certain amount of power to retain moisture, and the particles after rain tend to cake together and form a hard layer on the surface which makes it difficult for the penetration of young roots. Soils composed of very large amounts of fine sand are difficult to cultivate with any degree of success in times of drought as the water-holding capacity is not high.

Coarse silt forms a considerable proportion (30 to 40 per cent.) of the so-called loams, which are remarkable for their great fertility. Fine silt possesses the powers of cohesion and retention of moisture to a high degree, but too high a percentage of fine silt in the soil makes it difficult to work. Fine silt also shows some of the properties associated with suspensoid colloids.

The clay fraction of the soil exhibits true colloidal properties. It is unfortunate that the term "clay" has been used in such different senses by different workers. As Russell (*Soil Conditions and Plant Growth*, 1927) remarks: "The voluminous literature of clay is almost hopelessly complicated by the circumstances that different writers use the word in different senses; ceramic workers apply it to all material less than 0.1 mm. in diameter, while soil investigators in the United States restrict it to material below 0.005 mm. in diameter, and, in Great Britain, to that below 0.002 mm. in diameter. Modern supercentrifuges have effected still further division, separating out an 'ultra-clay,' the particles of which fall below 0.001 mm. in diameter in which the clay properties are much intensified."

Clay, when mixed with water, swells considerably, becomes viscous and sticky and can be moulded into different shapes. Heat is evolved in this process and absorbed on drying, when considerable shrinkage occurs. Clay particles exhibit the Brownian movement when suspended in water, and the particles carry a

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negative charge. The addition of acids causes flocculation (see below). The change, however, is only temporary in character. If the flocculating agent is removed by washing, deflocculation takes place. Alkalis also cause deflocculation. On heating, clay permanently loses its characteristic properties.

In general terms clay consists of a colloidal complex containing silica, aluminium and ferric oxides together with a certain number of bases. The chemical properties of clay are of great importance, for it takes an active part in certain chemical reactions in the soil. According to Blanck and Preiss (1921), 80 per cent. of the total clay analysed by them approximated closely to the theoretical complex: $\text{Al}_2\text{O}_3, 2\text{SiO}_2, 2\text{H}_2\text{O}$. The remaining portion, which consisted of coarser grades, showed a gradually increasing silica content.

The constitution of the inorganic colloids, which are collectively termed clay, has provoked a very considerable volume of research in the last few years. Bradfield (1923) has examined the colloidal material from a heavy type of clay, and his analyses appear to indicate that the natural colloid is possibly composed of the broken-down products of decay, *i.e.*, colloidal silica, alumina and ferric hydroxide. But comparison of the natural product with a synthetic mixture of these three substances showed very different behaviour to cataphoresis and electrolytes. It would seem that the naturally occurring colloidal complex consists in the main of readily decomposable alumino-silicates.

Robinson and Holmes (1924), in an investigation of the chemical composition of soil colloids from forty-five different soils in the U.S.A., found that only a small proportion of the material consisted of finely divided unweathered mineral fragments, and that the major portion was composed of material that behaved as an intimate mixture of complex alumino-silicates and not as an intimate mixture of separate constituent oxides, and thus are in general agreement with the investigations of Bradfield.

The colloidal coating on the surface of the mineral soil particles is largely made up of a gel composed of complex alumino-silicic acids containing reactive groups saturated to a greater or less extent by bases. Ganssen (1923) has put forward the hypothesis that it is possible to deduce the solubility of the nutrient materials

in and the manurial needs of the soil from the molecular proportion of silica, alumina and bases in the colloidal clay of the soil. Thus, if the molecular ratio: $\text{SiO}_2 : \text{Al}_2\text{O}_3 : \text{bases (Na}_2\text{O, K}_2\text{O, MgO, CaO)}$ be 3 or over : 1 : 1, the soil is absorptively saturated or neutral and an active exchange of bases is possible between the salts in the soil solution and the colloidal clay. This soil will be fertile. If, on the other hand, the ratio be 3 or more : 1 or less than 1, the soil will be acid and the salts of the soil solution will be decomposed by the adsorption of kations, and the soil will no longer be fertile. Tacke and Arnd (1923) investigated a number of heavy clay soils from this standpoint, and in no case found that the known manurial needs of these soils could be correlated with the molecular ratio of $\text{SiO}_2 : \text{Al}_2\text{O}_3 : \text{bases}$ in the manner suggested by Ganssen.

Recent investigations, which will be considered in detail, appear to show that clay is built up like a mineral salt of acid and basic radicle, *i.e.*, of anion and kation, and therefore, like salts, can enter into double decomposition with other salts with an equivalent exchange of kations. Bradfield (1923) found that the same amount of a suspension of an acid colloidal clay was necessary for the neutralisation of equivalent quantities of calcium or sodium hydroxide. The conductivity curves were of the type generally obtained in titrating a strong base with a weak acid and exhibited well-defined breaks showing that definite acids had been neutralised. He therefore concluded that the reaction between acid colloidal clays and strong bases was that of ordinary neutralisation between weak acid and strong base, and therefore any theory of physical adsorption of kations was unnecessary. Thus, when a soil is treated with a solution of a neutral salt, double decomposition takes place, so that the amount of kation of the neutral salt disappearing in the reaction from the solution is equivalent to the amount of kations that leave the colloid and appear in the solution. Sharp end-points may be obtained in conductivity titrations of clays or soil, provided the titrations are carried out in such a manner as to make possible the detection of the end-point in the neutralisation of a weak acid and strong base. This has been effected by the slow addition of the

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clay suspension to a fixed quantity of strong base, and not *vice versa*, as has usually been the case in previous investigations. The nature of the curves showed that definite weak acids were being neutralised in the process. Further support for this was obtained by the fact that almost equivalent amounts of different bases were needed for the attainment of neutralisation, as was shown by the breaks in the titration curves.

Bradfield considered that the view of Salter and Morgan (1923) of the reaction between clay and alkali being one of physical adsorption was untenable in the light of his results, and that the interaction of clay and alkali was that of a weak acid of high molecular weight with a strong base. In a second important communication, Bradfield (1924) described the preparation of a series of suspensions of the same colloidal clay material containing varying proportions of water, and determined the hydrogen-ion concentration by the hydrogen-electrode method. The curve obtained by plotting hydrogen-ion concentration against dilution was very similar to that showing the hydrogen-ion concentration of solutions of acetic acid at a variety of dilutions.

The particles of a clay suspension carry a negative charge. Dayduff and Hoagland (1924) have made a series of measurements of cataphoresis of an acid clay over a range of pH 2.1 to 12.7. The results of their experiments appear to show that throughout this range, which varied from markedly acid to markedly alkaline, the charge on the particles remained unaltered and negative, *i.e.*, they always moved to the kathode. When clay is brought into a salt solution, exchange of kations is rapid, a result that suggests that the kations are situated at the surface of the clay particle. Further confirmation of this view comes from the fact, as has already been stated, that clay suspensions in water develop a negative charge owing to the escape of some of the kations into the dispersion medium. Hissink (1923) therefore suggested that when the clay particle is suspended in water it is surrounded by a Helmholtz double electric layer, the negative layer (composed of anions) being in the interior and the positive layer (composed of kations) at the exterior.

More recently Gile (1925) has put forward the view that the

dispersed clay particle is like a loose mosaic made up of stones of different sizes with internal pore space. The silica and organic matter are scattered throughout this mosaic-like structure so as to preserve homogeneity of the complex and at the same time to form most of the bounding surfaces exposed to a liquid. The replaceable bases are held on this surface, but some of the kations diffuse into the liquid and form a Helmholtz double layer. Bradfield's views, however, are very much more in accordance with the known facts. Moreover, it is possible to extend his views to the production of so-called "alkali soils."

Alkali Soils.—Normal clay shows a preponderance of calcium ions, but these can be replaced by either sodium or hydrogen ions, when the soil is said to be an alkaline or sodium clay in the first case and acid clay in the second. The production of a sodium clay is frequent in arid climates through lack of rain and when the drainage is poor. Its most important chemical characteristic is the fact that in the presence of carbon dioxide it forms sodium carbonate, which rapidly tends to make the soil viscous and impervious to water, and at the same time is directly toxic to vegetation. It is also more highly ionised than a calcium clay and carries a heavier electric charge.

It is clear from Bradfield's results that when the soil is treated with a solution of a neutral salt, the soil colloids will undergo reciprocal change to that of the solution. Should the concentration of the neutral salt solution be greater than that which would be in equilibrium with the kations adsorbed by the soil colloids, the decrease in concentration of the kation of the neutral salt in the solution will be accompanied by an increase in the concentration in the colloidal phase. Continual treatment with the neutral salt solution will eventually lead to a saturation of the colloidal phase with the kations of the neutral salt. Thus, in the case of the alkaline clays the adsorbed calcium of the colloidal phase is replaced to a certain extent by sodium. Provided that a large excess of sodium ions are present, this calcium-sodium colloidal complex will be stable. Should, however, the excess of sodium be removed by washing, hydrolysis of the complex will occur with the formation of sodium hydroxide, and the latter is rapidly con-

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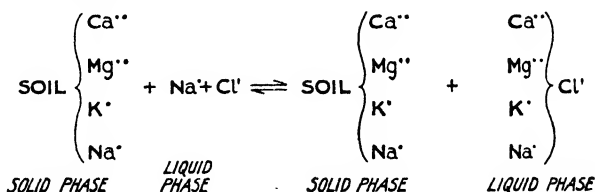
verted into sodium carbonate by the carbon dioxide of the soil atmosphere.

A somewhat similar view was put forward some years ago by the Russian worker, Gedroiz (1912, 1914, 1916), but perhaps, as Russell suggests, owing to the fact that his papers were written in Russian, their true significance and importance was never understood. Cummins and Kelley (1923, see also Kelley and Thomas, 1923, and Kelley and Cummins, 1921) have made a particular study of alkaline clays in California with special reference to the formation of sodium carbonate. In one case they were able to convert an acid clay to an alkaline one by a single treatment with sodium chloride, followed by washing with water. They showed that the sodium replaced a part of the calcium from the soil silicates with great rapidity. When the concentration of the sodium in the solution was high, the calcium set free could be easily removed, and a relatively high degree of saturation with sodium could occur. The physical properties of the new sodium-silicate complex were so different from the original calcium complex that the entire properties of the soil mass were profoundly altered.

These sodium complexes are very much less stable than the corresponding calcium compounds. They are slowly hydrolysed in the presence of strong electrolytes to give sodium hydroxide, and this hydrate then reacts with the carbon dioxide of the soil atmosphere in the way described above.

The Exchangeable Bases of the Soil

Those bases which are instantaneously removed from the soil on treatment with a solution of a salt are termed exchangeable bases. There is a real difference between the exchangeable and other bases of the soil, for different treatments always remove the same amount of these bases. The process of exchange may be shown as follows :—



The actual quantities of the exchangeable bases found in different soils differ with the conditions of manuring, and the extent to which this exchange takes place varies with the equilibrium concentration of the entering kation (*i.e.*, of neutral salt used); thus the curve conforms to the general type of Freundlich's adsorption curves.

The elements calcium and magnesium predominate in neutral soils, whereas, in alkali soils, sodium and, to a lesser extent, potassium hold the dominant position. Again, in neutral and alkali soils, aluminium, manganese and iron are absent, but they may be present in acid soils.

The adsorbing materials of soils that are acid (*i.e.*, those soils the aqueous suspensions of which have a pH of less than 7.07) possess a lower quantity of exchangeable bases than those of neutral soils ($pH = 7.07$). These acid soils are said to be "unsaturated," and, in general terms, the greater the unsaturation the greater is their acidity.

The behaviour of unsaturated or acid soils with regard to their base exchange has led to considerable controversy. Unsaturated soils, when treated with solutions of neutral salts, give an acid reaction to the liquid; an acidity technically termed "latent" or "exchange" acidity. This acidity is due to a hydrogen-ion concentration that is higher than the real or, as it is sometimes termed, "active" acidity of the soil, which is the pH value exhibited by the suspension in water. The "total" acidity of the soil is represented by the amount of base that must be added to bring the soil to neutrality. Investigators who uphold a theory of physical adsorption in soils explain the exchangeable acidity as due to selective adsorption of the kation of the neutral salt, leaving the anion in solution, and explain the total acidity by the fact that the soil can adsorb basic hydroxide as a whole.

Gedroiz (1924) and Hissink (1924) have both pointed out that these various phenomena are susceptible of correlation if the adsorbing material of the soil is looked upon in the light of possessing a definite saturation capacity for kations, with the further assumption that the adsorbing material can take up hydrogen ions as well as metallic kations. In such circumstances the adsorbing

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material of a soil is always saturated with a constant amount of kations, the only difference being that in an acid soil the greater proportion of kations are hydrogen ions, whereas in a neutral soil a lower proportion of kations is present. An acid soil of this kind will give rise to a definite hydrogen-ion concentration when suspended in water, since this is the equilibrium concentration for the liquid phase in equilibrium with the adsorbed ions of the solid phase. When the soil is treated with a neutral salt solution, an exchange occurs between the kations of the neutral salt and the hydrogen ions of the solid phase, so that at equilibrium the latter possess a greater proportion of adsorbed basic kations and the liquid phase a greater proportion of hydrogen ions. In the case of salts of strong acids, *e.g.*, hydrochloric acid, the amount of titratable acid liberated is small, whereas with salts of weak acids, *e.g.*, acetic acid, a greater quantity of titratable acid is released, for a greater displacement of hydrogen ions can occur from the solid phase before the equilibrium concentration of the hydrogen ions in the liquid phase is reached, on account of the low dissociation of acetic acid.

It is possible that the soluble aluminium and iron salts that are present in acid soils, and which make their appearance in the acid solutions when these soils are treated with neutral salt solutions, are liberated by secondary reactions between the acid released and some constituent of the solid phase. It is also equally possible that they are present as exchangeable bases in the same way as other kations.

Hissink explained the adsorptive capacity of a soil as being due to the existence of colloidal acids which are ionised at the interface between the solid and liquid phase, so that an electrical double layer is formed at the interface with the negatively charged complex colloidal anion on the solid phase side of the double layer and with positively charged metallic or hydrogen ions on the liquid side. The practically instantaneous nature of the reaction lends strong support to the view that ionic exchanges of this nature are taking place.

Gedroiz (1925) has shown how the relation between many of the chief types of soil can be explained from a consideration of the

amount and nature of the exchangeable bases (kations) in soils. He divided soils into two chief types as to whether they do or do not contain adsorbed hydrogen ions in their complex. Soils of the second type, which he termed “saturated” soils, were again subdivided by him according to the nature of the exchangeable kation. The “tsernosen” type was mainly calcium with a little magnesium, and was relatively stable and little affected by the peptising or dissolving action of water. Where sodium was present as well as calcium, three possibilities existed: (1) Saline soils (solontshak) with dissolved sodium salts in their water, (2) alkaline soils (solonetz) in which the exchangeable sodium gave rise to sodium hydroxide by hydrolysis on account of the lack of appreciable amounts of dissolved neutral salts. Thus when the adsorbing complex was saturated with sodium it broke down readily under the influence of hydrolytic agents, and in the absence of calcium in the soil it gave rise to a third type (soloti) in which an actual loss of a portion of the adsorbing complex had taken place. In the presence of calcium carbonate, on the other hand, this loss did not take place, and the sodium was removed as sodium carbonate and the calcium entered the complex and left a soil little different from the original soil.

Soils which contain adsorbed hydrogen in their adsorbing complex owe their characteristics to the fact that the adsorbing complex, when especially unsaturated in bases, shows a particular trend to undergo decomposition and to give rise to a complex of a new type. This tendency, however, is by no means so great as is the case with alkali soils. Gedroiz recognised two types: (1) laterite soils, in which the adsorbing complex has a sharply defined destruction throughout its whole depth, with the simultaneous production of large quantities of alumina and ferric hydroxide and a diminution in the amount of silicic acid; and (2) podsols, in which the adsorbing complex has only suffered decomposition at the surface layers of the profile and the diminution of silicic acid is not so great as in the laterite type.

Page and Williams (1924), in an important communication dealing with the exchangeable bases of Rothamsted soils, have discussed the meaning of the term “saturated” in the light of

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modern views of the nature of the soil. The original should be consulted.

Anderson (1929) has studied in detail the changes in properties that different soil colloids undergo when the exchangeable bases are substituted by different kations. The data included determinations of swelling, adsorption of water vapour, wetting, moisture equivalent, cataphoresis and *pH* of seven widely different soils, when saturated with calcium, magnesium, potassium, sodium, hydrogen and the kation of methylene blue. The order of the kation effect on wetting and moisture adsorption was found to be :—

$$\text{Ca} > \text{Mg} > \text{Na} > \text{H} > \text{K} > \text{methylene blue kation}.$$

The decreasing order of effects on the remaining properties studied were :—

$$\text{Na} < \text{K} < \text{Ca} < \text{Mg} < \text{H} < \text{methylene blue kation}.$$

The influence of a kation on a property appears to be in direct proportion to its percentage of complete replacement, although it cannot be said that the data are particularly convincing on this point. The ranges of alteration of the different soil colloids correlate fairly well with the magnitude of the properties of the untreated or calcium saturated materials with the total content of exchangeable bases, and the exchangeable base-content is in a

measure dependent on the ratio : $\frac{\text{Si}}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$.

Flocculation

The flocculation of clay suspensions by electrolytes has already been briefly alluded to in general terms. The subject has received intensive investigation at the hands of soil physicists within recent years, and many of the conflicting statements on the matter have now been removed.

The hydrogen ion produces the greatest amount of flocculation compared with other univalent ions, while the coagulating power of salts of the same acid with different metallic radicles increases with the valency of the kation.

Gedroiz (1924) has reinvestigated the work of Hall and Morrison (1907), and has shown that the conflicting results of these authors can be readily explained by the very great variations found in the physical state of suspensions of clays, depending on the kation with which they are saturated. For example, clay saturated with calcium quickly settles to a compact sediment, whereas clay saturated with sodium ions gives rise to a voluminous jelly-like mass. Wiegner (1924) considered that a satisfactory explanation of the phenomena can be found in the variations in the degree of saturation of the kations in the clay or of the neutral salts which he used. The nature of the electrical double layer and the way in which it is influenced by the hydration of the ions in the outer layer play an important part. Thus, when the mobile ions of the outer layer are highly hydrated, they are unable to approach near to the inner layer. In such circumstances the distance between the two layers is increased and the potential of the inner layer increases. The greater the potential, the greater the stability, so that the expected stability of a series of clays would be in the order: Hydrogen clay, caesium clay, rubidium clay, potassium clay, ammonium clay, barium clay, strontium clay, calcium clay and magnesium clay.

It will be seen from this that the base exchange by the process of altering the predominant ion in the outer layer will materially alter the stability of the clay. It would be expected on these grounds that the most effective precipitating ions would be those which are least hydrated, since they would be able to get nearer to the inner layer than the more heavily hydrated ions, so decreasing the potential. Thus the difference between univalent and bivalent ions not only depends on their charge, but also on their degree of hydration.

The effect of the calcium ion on flocculation is of great importance to agriculture, and the behaviour of calcium hydroxide is of considerable interest in this connection. For example, such calcium salts as the nitrate or chloride produce a greater amount of flocculation of kaolin or silt than the hydroxide. This is due to the fact that the —OH ions of the hydroxide increase the negative charge on the particles. Similarly *dilute* suspensions of

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clay behave in the same way, but if the suspensions are greater, then the hydroxide produces a greater effect than other calcium salts. This "abnormal" flocculation of clay as it is called has received intensive investigation at the hands of Comber (1920, 1921, 1924) and Mattson (1922). Comber advanced the explanation that the clay particles were composed of a solid core surrounded by a coating of an emulsoid gel-like silica. The calcium hydroxide and silica reacted to produce a bulky precipitate which brought down the clay. When washed, the precipitate suffered hydrolysis and passed back into suspension once more. Comber distinguished three types of flocculation of clay :—

1. **Normal Flocculation.**—This resembles the behaviour of any other electro-negative suspensoid.

2. **Indirect Flocculation.**—Here the added electrolyte reacts with the clay and brings into solution other substances which cause flocculation.

3. **Abnormal Flocculation.**—This is caused by the reaction between the added substance which brings about flocculation [$\text{Ca}(\text{OH})_2$] and the emulsoid gel covering the surface of the clay particle.

Mattson preferred to consider this abnormal flocculation as being due to the preferential adsorption of $-\text{OH}$ ions. The charge on the particles was thus raised and this brought about an increased adsorption of calcium ions. Since calcium is a bivalent element, he made the further assumption that each ion of the metal was able to hold two clay particles together, and therefore on this account tended to bring them down. The explanation, however, loses a great deal of its force when it is remembered that sodium can behave in the same way.

The Organic Component of the Soil—Humus

The surface soil is always darker in colour than the subsoil. This is due to the presence of dark-brown or black material of vegetable origin. The soil organic matter is a heterogeneous collection constantly undergoing change. It approaches a condition of homogeneity when its composition reaches a certain

equilibrium, and it becomes more or less incorporated in the soil. This homogeneous mass is often spoken of as *humus*. The organic matter is an essential constituent of soil. It supplies a rich store of energy, making possible the existence in the soil of an abundant and flourishing microflora and microfauna.

Two types of soil may be distinguished in this connection : (a) mineral soils, in which the organic matter is comparatively low ; and (b) humus or peaty soils, in which it forms the greater portion.

The organic matter of the soil is derived from a great many different sources, such as residues of cereal and root crops, straw, animal excreta and the remains of plants. By far the larger portion of organic matter that reaches the soil is composed of carbohydrate and protein. These organic residues are acted upon by the micro-organisms of the soil, and by a series of complex biochemical reactions are converted into simpler products. Of all the products produced, humus has the least transitory existence in the soil, but it also ultimately suffers change and disappears.

It is unfortunate that there is a great lack of uniformity in applying the term "humus." Some investigators apply the name to the total organic content of the soil, while American investigators give the term to that part of the organic matter soluble in weak alkali. Odén (1919) classified the alkali-soluble fraction of the soil organic matter into three parts and termed the insoluble portion "humin." Of the alkali-soluble portion, a fraction is precipitated by the addition of acid, while brown water-soluble bodies, acid in nature, remain in solution ; these latter are termed "fulvic acid." A part of the precipitated fraction is soluble in alcohol and is called "hymatomelanic acid," while the residue insoluble in alcohol is termed "humic acid." It is this humic acid that has received the greatest amount of investigation.

In its general properties humus behaves as a colloid. The humin of the humus complex is not capable of sol-formation, but humic acid behaves as an emulsoid, and its hydrosols are relatively insensitive to the action of electrolytes. It can, however, be flocculated by electrolytes, especially by those electrolytes with polyvalent ions (*e.g.*, Ca) if they be sufficiently concentrated.

Humic acid is an electro-negative colloid, but the charge can suffer reversal. It is said to be electro-positive in alcohol. The humic acid fraction of the humus probably exists in the soil in the form of salts, especially with calcium, whereas it is probable that the humin fraction is more or less saturated by adsorption of various salts and other substances.

The presence of humus has a profound influence on the tilth of the soil. The combined action of humus and calcium carbonate

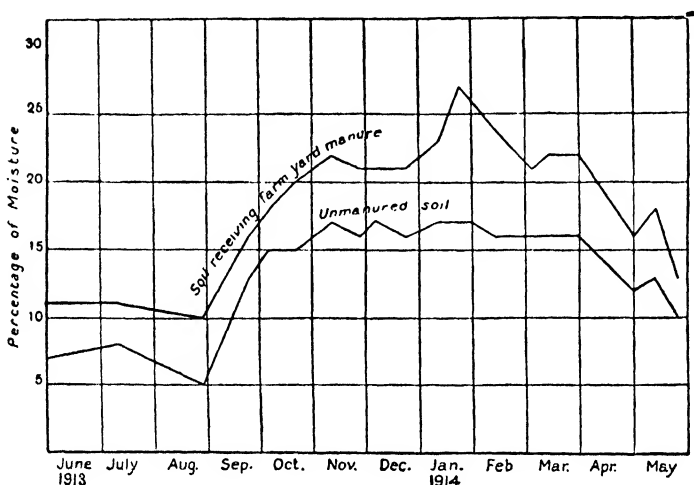


FIG. 1.—Curves showing the percentage of water in two soils on adjacent plots, one of which annually receives farmyard manure while the other does not. (After Russell, *Soil Conditions and Plant Growth*.)

helps to bring about this condition in heavy soils, and is very useful in this respect in agricultural practice. It also plays an important part in sandy soils and helps to give these "body" and prevents excessive drainage. The presence of humus also improves the water-holding capacity of the soil near the surface and makes the moisture available for the crop for a longer time. It is only where the proportion of humus is so high that the soil is peaty that water-logging occurs. The effect of humus on the water-content of mineral soils is illustrated in Fig. 1, which

shows the water-content of adjacent plots on the Broadbalk Field, Rothamsted, one of which receives farmyard manure annually, while the other receives no organic manure. It will be seen from the nature of the curves that the former contains about 5 per cent. more moisture than the latter.

The Chemistry of Humus.—The present position of the chemistry of humus is still in a highly confused state. This is not surprising when the complexity of the subject is considered. It has been known from the eighteenth century that organic matter is extracted from the soil by alkali after a preliminary treatment with dilute acid and that the dark-coloured extract could be precipitated by acid. A number of fractions have been isolated from the soil and given names and definite chemical formulæ, apparently on the assumption that these were homogeneous bodies. The reverse is the true situation. Such names as ulmin, humin, ulmic, crenic, apocrenic and geic acids still make their appearance from time to time in the literature. The matter will be considered here from the standpoint of Odén's classification.

Odén (1919–20) has shown that humic acid possesses definite electrical conductivity and gives true salts with alkalis. He concluded that it was tetrabasic and gave to it the formula $C_{60}H_{52}O_{24}(COOH)_4$, and stated that its molecular weight was of the order of 1350, and that no nitrogen was present. The latter statement is not in accordance with the observations of Page, who always found a constant amount of nitrogen (5 per cent.) which could not be removed by any process of fractionation.

The mechanism of formation of humus in the soil has led to endless controversy and is not yet settled. Two main views are prevalent on the subject: that it is formed (*a*) from carbohydrates, *e.g.*, cellulose, and (*b*) from the lignin of the ligno-cellulose complex.

Berkley (1920) found evidence for the presence of hydroxymethylfurfuraldehyde in dunged soil and rotting straw in which humus was being produced, and suggested that humus was formed in the soil in two stages:—

I. Carbohydrate (cellulose, etc.) + amino-acids = hydroxymethylfurfuraldehyde.

II. Hydroxymethylfurfuraldehyde + amino-acids = humus + furfuraldehyde.

The evidence on which this view is based is very slender. The furfural compound may well have been merely a transitory product in the oxidation of pentosans and has nothing to do with the production of humus.

Eller and Koch (1920) from their experiments *in vitro* on the oxidation of phenols in alkaline solutions supposed that humus is formed from the oxidation of phenols in the soil. The phenols are considered to be derived from hexoses by the elimination of water.

Marcusson (1921, 1922, 1923, 1924, 1925, 1926) is the chief upholder of the carbohydrate hypothesis for the origin of humus in the soil. He suggested that the carbohydrates in the plant residues are first converted into furfural, and this through the formation of a paradifurfurane ring is eventually converted into benzenoid groupings. His views have from time to time undergone considerable modification, and they will be considered from the point of view of the lignin hypothesis of humus origin.

It was suggested by Fischer and Schrader (1921) that humus and coal were formed from the lignin component of the ligno-cellulose complex. A considerable amount of evidence is now forthcoming to support this view. Schrader (1922) heated isolated lignin with a solution of sodium hydroxide to 200° C. under pressure, and found that it was almost completely oxidised to a humus-like substance. Experiments conducted over a prolonged period at ordinary temperatures with a 5N solution of sodium hydroxide in the presence of oxygen also showed that lignin underwent oxidation with loss of methyl groupings. In an atmosphere of nitrogen the oxidation proceeded more slowly. Schrader suggested that the part played by the sodium hydroxide in this reaction is fulfilled in nature by lime or ammonia, whilst bacterial action may also be of importance.

Bray and Andrews (1924) during a study of bacterial decay of wood found that the cellulose had almost disappeared in three years, whereas the lignin had suffered little diminution in amount. Toit (1924) could find no correlation between either cellulose or

pentosan destroyed in the soil and humic matter produced, but in all cases the disappearance of lignin was paralleled by the production of humic matter. Pure carbohydrates (cellulose, sugars, etc.) when decomposed by soil organisms completely disappeared and were not attended by the production of humic matter.

Tropisch and Schellenburg (1924) have studied the products of the dry distillation of humic acid prepared from coal in relation to its interaction with potassium hydroxide, nitric acid, sulphuric acid, and chlorine. The reactions showed that relatively simple aromatic substances were readily formed from humic acid and that humic acid and lignin behaved in a similar way in this connection. On the other hand, cellulose, glucose and artificially prepared humic acid made from sugar behaved somewhat differently. From these experimental observations they concluded that humus in the soil is formed from lignin, and not from cellulose or other carbohydrate. Fischer and Tropisch have shown that the behaviour of lignin on hydrogenation with hydriodic acid under pressure and also on treatment with alkali under pressure was very similar to the behaviour of humus, whereas the behaviour of cellulose is very different. Tropisch (1924) pointed out that the occasional presence of cellulose in coal is no proof that coal is formed from cellulose. It is far more difficult to account for the survival of the cellulose if coal be formed from it by heat and pressure than to suppose that the cellulose disappears under the influence of micro-organisms. In the latter case the local occurrence of aseptic conditions would account for its survival. Grosskopt (1926) has also brought forward evidence to show that humus is formed from lignin and not from carbohydrate. He investigated the successive layers of humic soil in a pine wood and found that there was a marked correlation between loss of lignin and formation of humus.

Further support for the lignin hypothesis of humus origin is given by the recent work of Barton-Wright and Boswell (1929), who have shown that the action of the saprophytic fungus *Merulius lachrymans* (the cause of dry-rot in wood) on spruce wood is to consume the mannan, galactan and cellulose and to

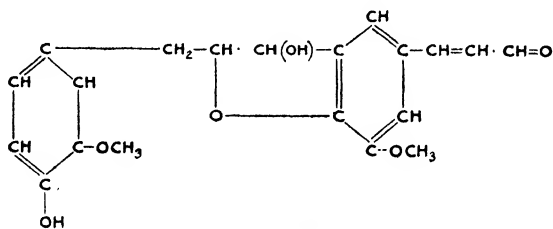
leave the lignin and hemicellulose fractions. It is curious that the connection of these hemicelluloses with humus formation has never been investigated. They are acid bodies, soluble in dilute solutions of alkali, and on hydrolysis give rise to pentoses and hexoses. On distillation with 12 per cent. hydrochloric acid they give furfural and a quantitative yield of carbon dioxide showing the presence of "uronic" acids in their molecule (see O'Dwyer, 1926; Dorée and Barton-Wright, 1927). Thaysen and Bakes (1927) claimed that the action of aerobic bacteria on rotting straw is to give the carbohydrate fraction of humus by the decomposition of hemicelluloses, but apparently their use of the term hemicellulose in this connection is synonymous with pentosans.

The action of fungi on wood is complex. In the "brown" rot type of decay, such as is brought about by *Merulius lachrymans*, the lignin remains unattacked and the fungus attack is confined to the carbohydrate components of the wood. In the "white" rot diseases, on the other hand, it is usually stated in mycological literature that the carbohydrate portions remain undisturbed and the lignin is removed. The evidence of the latter statement is very slender, though quantitative experiments on *Trametes pini* have been described as confirming this statement. Campbell and Booth (1929) have investigated the decay of the heartwood of Silka pine by *Trametes serialis*. Decay was allowed to proceed for three months and four months respectively. Analysis showed depletion of the cellulose and pentosans and the lignin was only affected to a slight extent. Like Barton-Wright and Boswell, they found an increase in the solubility of the decayed wood in solutions of sodium hydroxide. Campbell and Booth considered that there is a marked similarity between acid hydrolysis of wood with dilute mineral acids and the action of fungi. Both give rise to a product soluble in 1 per cent. sodium hydroxide solution which cannot be determined as pentosan, cellulose or lignin, and the only objection that can be found for this suggestion is the fact that apparently there is a greater solubility of decayed wood in alkali. But when the water-soluble fraction of decayed wood is added to the cold water-soluble fraction of wood hydrolysed by acid, and this subtracted from the total amount of decayed wood

soluble in dilute alkali, there is a strict comparison to be found between them. This is a legitimate procedure, since in the hydrolysis of wood with dilute acid the water-soluble portion is removed, but in the fungal-decayed material it still remains.

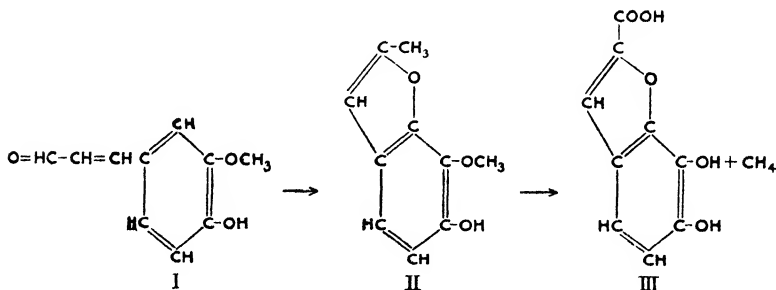
Odén and Lindberg (1926) analysed peats of various ages and found that the cellulose gradually disappeared, but the sum of the lignin plus the humic material remained more or less constant, and as the lignin content fell, that of the humus took its place.

On a consideration of Klason's views of the constitution of his so-called α -lignin, a body of formula $C_{20}H_{20}O_8$, to which he assigned the constitution



α -LIGNIN

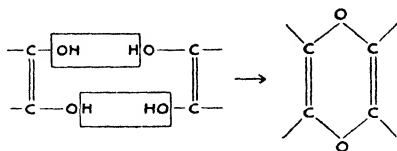
—i.e., a substance derived by the condensation of two molecules of coniferyl aldehyde—Odén and Lindberg supposed that the coniferyl aldehyde part of the molecule (the right-hand portion of the constitutional formula given above) of α -lignin is converted into furfurane derivatives by internal condensation:—



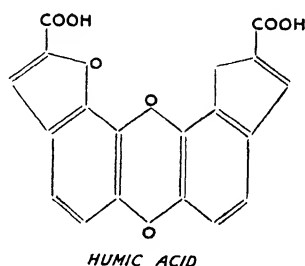
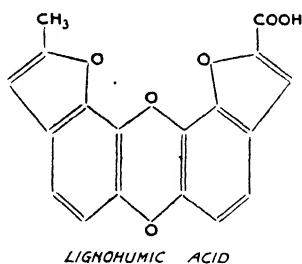
By simultaneous oxidation and reduction of (II), acid (III) would be produced containing two phenolic hydroxyl groups in

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the ortho-position. In an oxidising medium, o-dihydroxyphenols of this type readily undergo condensation into quinonoid bodies :—

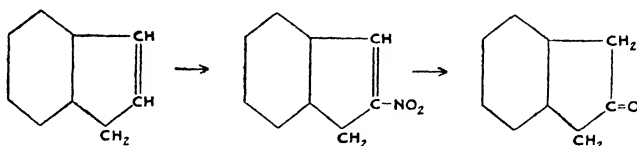


The production of a ring compound of this type is considered to be characteristic of the humification process, which involves the production of strongly coloured bodies from colourless ones. Odén and Lindberg suggested structural formulæ for humic and lignohumic acids involving the combination of units of structures—I, II and III. Thus they formulated a lignohumic acid built up from (II) and (III), and a humic acid built up from two units of (III) :—

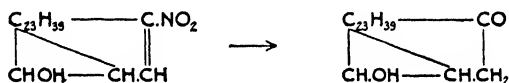


It is in the highest degree improbable that these reactions actually occur in the formation of humus in the soil. In the first place it is generally assumed by all humus workers that lignin is composed of condensed aromatic nuclei. It is very unlikely that this is the case. The investigations of Dorée and Hall (1924) and Dorée and Barton-Wright (1929) on the lignosulphonic acids isolated from spruce wood in the commercial production of wood pulp showed that the isolated product after purification resembled in its properties and reactions hydroaromatic rather than aromatic compounds. For example, on heating lignosulphonic acid with 5 per cent. nitric acid, an orange-red nitro-body was isolated.

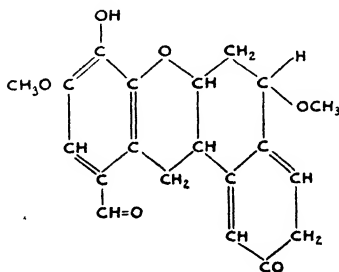
This, on reduction with zinc dust and hydrochloric acid or magnesium powder, gave rise to a nitrogen-free ketone. The formation of a nitrogen-free ketone from a nitro-compound is a reaction characteristic of hydroaromatic and not of aromatic bodies. Thus indene on treatment with nitric acid gives a nitro-body, which on reduction yields the nitrogen-free ketone β -hydrindone :—



Similarly the complex alcohol, cholesterol on nitration, gives a nitro-derivative, and this on reduction forms the nitrogen-free ketone dehydrocholestanol :—



In a similar way it was shown by Dorée and Barton-Wright (1927) that alkali lignins, *i.e.*, products isolated from wood by treatment with sodium hydroxide under pressure, behave in the same way. They were able to isolate from spruce wood a body of molecular formula $C_{20}H_{20}O_6$, to which they gave the name meta-lignin. This up to the present is the simplest lignin that has been isolated from woody tissues. This body on nitration and subsequent reduction gave a nitrogen-free ketone, and these investigators from this and other reasoning assigned to it the ideal formula



which is a constitution based on condensed hydroaromatic nuclei.

Klason's views on the constitution of lignin are scarcely in accord with the known facts, and any theories of humification based on his ideas should be regarded with suspicion.

An important argument advanced by Marcusson (1925) against the lignin hypothesis of humus origin is that sphagnum peat contains over 50 per cent. of humic acid, although the probabilities are that sphagnum contains little if any lignin. This is certainly a serious objection, and the whole problem of humus formation from sphagnum requires serious reinvestigation. Marcusson has now apparently formed the opinion that both carbohydrates and lignin take part in humus formation. The work of Thaysen, Bakes and Bunker (1926) is of interest in this connection. They considered that microbiological activity is insufficient to eliminate all the cellulose present in plant tissues decaying to form peat. The production of peat takes place under waterlogged conditions. The presence of fungi are therefore eliminated, and they cannot play a part in the matter. Thaysen, Bakes and Bunker have been able to isolate humic compounds from typical peats which consist of two distinct types. One of these yields a chlorine derivative very similar to the humus of ordinary soil, and is apparently related to lignin, whilst the chlorine derivative of the second type is closely related to the chlorine derivatives of the so-called artificial humus compounds which are produced by the action of inorganic acids on carbohydrates or from cellulose fibres by decay. The presence of the latter in peat may be due to the decomposition of cellulose not destroyed by microbiological activity.

Soil Moisture

The chief source of soil moisture is rain. A portion of this runs into the subsoil and may be carried away by drainage, and a portion is retained by the surface layers. The excess water which sinks into the subsoil is spoken of as gravitational water. Some of the water in the soil is retained as fine films round the

soil particles or in the minute crevices that are present, and this is termed capillary water. The so-called hygroscopic water is the portion retained by the soil colloids.

The problem of soil moisture is very much more complex than the simple classification given above would lead one to suppose, and the complexity of the problem has only been realised within recent years. On the older view the water was considered to be in the form of fine films round the soil particles. But the advent of investigations which showed that the soil is a complex colloidal system has materially altered the older point of view.

It was found in 1916 by Bouyoucos and McCool that the lowering of the freezing point of a soil increased approximately in geometric progression as the moisture content decreased in arithmetical progression. This indicated, contrary to general belief, that the concentration of the soil solution (see below) is not inversely proportional to the moisture content of the soil. The hypothesis which was advanced to explain these results assumed that the soil renders part of the soil moisture inactive as a solvent, thereby causing the soil solution to become more concentrated; the percentage moisture of the soil not indicating this state of affairs. The amount of "inactive" or "free" water was measured by a dilatometer. Using this method, Bouyoucos (1921) formulated a new classification of soil moisture :—

Gravitational.

Free.

| | | | | |
|--------|---|---------------------|---|--|
| Unfree | { | Capillary absorbed. | { | Water of solid solution or water of hydration. |
| | | Combined | | |

The "free" water was the moisture which froze for the first time at -1.5°C . This temperature was chosen rather than 0°C ., because the freezing point of the soil is not 0°C ., but a little lower than this value. The "capillary absorbed" water was that which froze finally at the supercooling of -4°C . The "combined" water was the term given to that fraction which did not freeze at all. Bouyoucos considered the older classification of

gravitational capillary and hygroscopic water to be too empirical and general to be of exact scientific value and its origination to be due to the fact that the older workers supposed the soil to be made up of a mass of particles over the surface of which the moisture spread. Parker (1922) took exception to Bouyoucos's expression "unfree" water (which the latter found to vary between 1.2 in coarse sand and 22.84 in clay), and claimed that the dilatometer is not capable of distinguishing between and measuring different forms of water in the soil and that soils as a whole do not contain a considerable percentage of water which does not act as a solvent. This is a perfectly valid objection, and although the older classification leaves much to be desired, it still serves its purpose.

It has already been seen that the soil is complex in nature and its relationship with water is both intricate and intimate. Thomas (1921) has shown that the vapour pressure curves for different soils are in the nature of rectangular hyperbolæ over a wide range of moisture content. This means that the vapour pressure is proportional to the reciprocal of the moisture content. The position of each curve depends almost wholly on the texture of the soil in the absence of dissolved material.

Keen (1914, 1922) found that there were no sharp breaks in the curves obtained for the evaporation of water from the soil, so that it would appear that the fractions postulated by Bouyoucos are not sharply separated from one another. The evaporation of water from sand, silt, china clay and ignited garden soil are relatively simple phenomena, and follow the known laws of diffusion and evaporation. Evaporation from the soil, however, is a more complex phenomenon, and there is some factor present which operates in making the relation between the soil and soil water of a different and closer nature than is the case with sand, silt, etc. The complex nature of the evaporation from the soil is not due to the presence of soluble humus, for its removal does not appreciably affect the problem. When, however, the colloidal properties of the soil are destroyed the nature of the curve is quite different and becomes identical with that of sand and silt. Evidently it is the colloidal nature of the soil which is responsible for the result. The matter has been further investigated by Keen,

Crowther and Coutts (1926), who discovered that the evaporation of water from the soil was controlled by two factors depending on (1) the soil-water relationships, and (2) environmental conditions. The latter includes such factors as the diffusion of water vapour of the soil and bulk air movements set up by (a) the temperature gradient from bottom to top of the soil, (b) the cooling of the soil by evaporation, and (c) the lower density of moist air. Thus the environmental difficulties are great and not easily overcome in experimental work on this subject.

Briggs and McLane (1907) have endeavoured to find the relative force with which the different mechanical particles of the soil hold their water. The experimental technique employed was simple. The samples of soil were placed in metal cups and centrifuged at a rapid rate, a centrifugal force of 1,000 gm. being developed. The water was flung off the particles and a measure of their water-holding capacity was obtained.

The following values were recorded :—

| | Diameter of Particles. | Relative Water-holding Capacity. |
|---------------------|------------------------|----------------------------------|
| Coarse sand | 2.0–0.25 mm. | 1 |
| Fine sand | 0.25–0.05 mm. | 1 |
| Silt | 0.05–0.005 mm. | 12 |
| Clay | 0.005 and less | 57 |
| Humus | — | 57 |

It will be seen that the relative water-holding capacity increased from coarse sand to humus. Shull (1913, 1916, 1920) used the seeds of the composite *Xanthium* to ascertain the force with which the soil moisture was retained. The seeds were weighed and placed in soils of different moisture content and allowed to remain till equilibrium had been established and were then weighed again. Any increase in weight gave the amount of water taken up by the seeds. An endeavour was then made to measure the actual force exerted by the seeds to take up this water. The seeds of *Xanthium* possess testas which are semi-permeable, and the seeds were placed in different concentrations

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of sodium and lithium chloride solutions, the osmotic pressures of which could be calculated. The amount of water taken up in these solutions was then compared with the values obtained from the soil. Shull considered that the osmotic pressure of the solution from which the same amount of water was withdrawn as from the soil gave a measure of the force with which that soil held its moisture. In one case it was ascertained that an initial force of nearly 1,000 atmospheres had to be exerted by the seeds to remove water from the soil.

Absorption of Water by the Root

In the case of land plants it is only a special portion of the root that can absorb water and dissolved salts, namely, the root hairs. It is often stated that the function of the root hairs is to absorb water. This is certainly true, but they also possess another important function, inasmuch as they increase the area of absorption of the root.

The root hairs are delicate structures which grow out from the piliferous layer of the root. They have a more or less gelatinous coating on their walls by means of which they are able to adhere to the particles of the soil and make intimate contact with them. They do not ordinarily occur over the entire surface of the root. The terminal portion of the root, consisting of the root cap and the region of cell division and cell enlargement, does not bear root hairs. The older portions of the root are also devoid of hairs. Generally speaking, the root hairs are for the most part confined to a limited zone of about 1 to 4 cm. in extent near the tip of the root. (For a full review of the literature dealing with the structure and physiology of root hairs, see Farr, 1928.)

Till the important publication in 1918 by Thoday, it was considered that the root hairs withdrew water from the soil by virtue of their osmotic pressure. This, however, is not the case. The entrance of water into the root hairs depends upon what has been termed by Stiles (1922) the *suction pressure* of these cells. A consideration of this subject will necessitate a brief digression at this stage.

If a cell isolated from its surroundings be placed in water, water will enter and distend the cell wall. The wall is elastic, in that it is able to resist extension. The distended wall compresses the protoplasm and cell sap, with the result that the consequent hydrostatic pressure, usually termed the *turgor pressure*, is equal and opposite at any moment to the inward components of the tensions of the cell wall. This turgor pressure tends to force water out of the cell. When the swelling is complete and equilibrium reached, the turgor pressure completely balances the osmotic pressure of the cell sap. In this condition the cell is fully

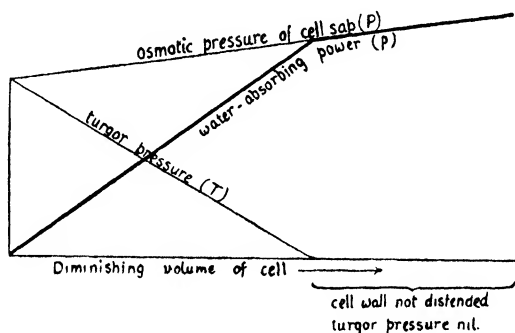


FIG. 2.—Curves illustrating the relationship between the osmotic pressure of the cell sap (P), turgor pressure of the cell wall (T) and suction pressure of the cell (p). (After Thoday, *New. Phyt.*)

turgid and it is in equilibrium with water, and for this reason is incapable of absorbing more water. This condition will last as long as either the osmotic pressure or the tension of the cell wall suffer no change. If this turgor pressure be represented by T, and the osmotic pressure by P, then under the conditions of equilibrium discussed above :—

$$P = T$$

or

$$P - T = 0$$

The value $(P - T)$ is known as the suction pressure of the cell. It is the part of the osmotic pressure left over to suck water into

the cell and gives a measure of the water-absorbing capacity of the cell. This suction pressure is fundamental in all processes connected with the water-absorbing power of root hairs. ✓

The connection between the osmotic pressure, turgor pressure and suction pressure of a cell is shown in Fig. 2. It will be seen from the nature of the curve that with diminishing turgor pressure there is a corresponding rise in the suction pressure.

✓In the case of a fully flaccid cell, the turgor pressure is zero. The osmotic pressure is equal to the suction pressure, and the water-absorbing power of a fully flaccid cell depends on its osmotic pressure. From this discussion it will be understood that the older view that it was the osmotic pressure of the cell which played an all-important part in the absorption of water is erroneous.

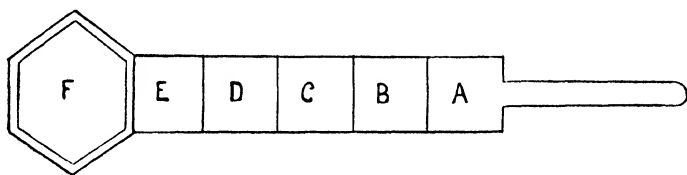


FIG. 3.—Diagrammatic representation of root hair, A, in connection with cortical cells B to E, and trachea F. (After V. H. Blackman, *New. Phyt.*)

Condition of Equilibrium between Adjacent Cells.—Since the water-absorbing power of a cell is dependent on its suction pressure, the question arises as to how far this will affect the situation between two cells in contact with one another. If these cells are represented by A and B, we have as the condition of equilibrium :—

$$(P_A - T_A) = (P_B - T_B)$$

If the value $(P_A - T_A)$ be greater than $(P_B - T_B)$, then A will absorb water from B, irrespective of the absolute values of P_A and P_B . It is quite possible, for example, to have B with a greater osmotic pressure than A, yet because the turgor pressure is also greater, it may be quite unable to absorb water from B, and, in fact, may yield water to it. Similarly, as V. H. Blackman (1921) has pointed out, if a root hair be in contact with a number of

cortical cells and finally a xylem vessel (Fig. 3), A, B, C, F and a gradient of suction pressure be established, water will enter the root hair A, pass into the cortical cells, and finally enter the trachea. A gradient of suction pressure all the way is required. On the assumption that the root hair A is exposed to water and has the highest osmotic pressure, the cells B to F having progressively lower pressures, water will still pass from A to F. As A takes up water, its absorbing power will fall below B, and B will begin to take up water from A. In the same way as the absorbing power of B falls below the osmotic pressure of C, C will take up water from B. As the cells of the root hair and cortex become more and more turgid and cease to have any absorbing power, F will be able to draw water from outside A, however low be its osmotic pressure, and the force with which this water will be drawn in will depend absolutely on the difference between the osmotic pressure of the solution external to A and the osmotic pressure of F. The presence of the intermediary cells can be neglected, though their interposition will naturally cause a reduction in the rate of flow. ✓

Measurement of Suction Pressure.—Two distinct methods have been described by Ursprung and Blum (1916, 1918, 1919, 1920) for the determination of suction pressure. ~ Since the suction pressure represents the osmotic pressure of the cell minus the turgor pressure of the cell wall, Ursprung and Blum hit on the idea of directly employing plasmolysis for this purpose. ✓ The actual estimation was carried out as follows: In the first stage it was necessary to measure the volume of the cell, and since this could not be effected in water or salt solutions, it was carried out in liquid paraffin. The second stage consisted in finding a cane sugar solution that just did not change the volume of the cell, and, lastly, one that just did. The mean of the two values gave the suction pressure of the cell. ✓

The second method consisted in determining the concentration of a solution which contained some substance to which the cell membrane was impermeable and caused no change in the volume of the cell. Here the value of the suction pressure would be zero, and if P' represents the osmotic pressure of the external solution,

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P the osmotic pressure of the cell sap, and T the turgor pressure of the cell wall, we have :—

$$\begin{aligned} P - P' - T &= 0 \\ \text{or} \quad P' &= P - T \quad . \quad . \quad . \quad . \quad . \quad (a) \end{aligned}$$

If S represents the suction pressure, when the cell is placed in distilled water :—

$$S = P - T \quad . \quad . \quad . \quad . \quad . \quad . \quad (b)$$

From equations (a) and (b) it follows that P' and S are both equal to (P - T) and the value of T is the same in both equations, thus :—

$$S = P'$$

Hence the suction pressure of a cell is equal to the osmotic pressure of some non-penetrating solution in which the cell remains unchanged in volume. As a matter of fact, in actual practice, it is better to use weight rather than volume for the estimation. ✓ To find the suction pressure of the cells of some given tissue by this method, all that is necessary is to take varying concentrations of cane sugar solution and immerse equal weights of the tissue in them for a given time (usually two hours). The tissue is then removed and weighed. The osmotic pressure of the concentration which brings about no change in weight of the tissue gives the value of the suction pressure of the cells.

More recently Molz (1926) has devised a very simple method of estimating suction pressure. Cane sugar solutions of varying concentrations were placed in small bottles of 20 c.c. capacity. The plant organ, or part of the organ, after severance from the plant was placed in paraffin oil, in which it could remain for several hours without injury. Appropriate strips were then cut from the material under paraffin. The strips were so cut that their length could be determined exactly by means of a scale on a microscope slide and a micrometer eye-piece. The length of the strips was determined and the paraffin was quickly removed with a piece of filter paper, and they were placed in the bottles with cane sugar

solution for one to one and a half hours. The lengths were measured again in the strength of cane sugar solution in which the strip had been immersed. The osmotic pressure of the cane sugar solution which caused neither increase nor decrease in length of a strip gave the suction pressure of the tissue.

Suction Pressure and its Magnitude.—The first really adequate measurements of suction pressure were made by Ursprung and Blum (1916, 1921). Working with the roots and leaves of *Fagus sylvatica* they discovered considerable differences in the value of the suction pressure in different parts of the same organ. In general terms the suction pressure rose from the guard-cells, through the spongy tissue to the palisade parenchyma in the leaf. In the stem, the gradient of pressure was from within outwards, and in the root from the piliferous layer inwards; in fact, the reverse of the stem. They found in *Vicia faba* and *Phaseolus vulgaris* that the suction pressure of the cells of the roots in both species showed a steady increase from the piliferous layer as far as the innermost layer of the cortex which abutted on the endodermis, but the suction pressure of the latter was very much less than that of the cortical tissue, but higher than that of the pericycle. These investigators explained this curious anomaly by assuming that the suction pressure on the two sides of the endodermal walls was not the same, and that in their experiments the mean of the two values was necessarily taken.

According to Ursprung and Blum (1921), the suction pressure of root cells quickly accommodated itself to the osmotic pressure of the solution to which they were transferred. Bean roots, for example, germinated in sawdust, showed a suction pressure of 1.4 atmospheres, but when they were transferred to a 0.65 per cent. cane sugar solution of osmotic pressure 5.3 atmospheres, the value of the suction pressure rose to 5.7 atmospheres in just over three days. It is therefore possible that the suction pressure of root cells is a measure of the forces of the soil which resist the withdrawal of water, being just greater than these.

Blum (1926) has investigated the suction pressure of alpine plants. Here the aerial regions were always found to give higher values than the root tissues. Seasonal changes, such as rainfall,

caused variations in these values. Again, the values obtained for the floral organs were very high. Of the various factors influencing the suction pressure of the cells, soil moisture was found to be the most important. Any decrease in the soil moisture led to an increase in atmospheric humidity, causing strong modifications in the suction pressure. Molz has also shown that soil moisture and atmospheric humidity are important external factors which influence the suction pressure of cells. For example, strong rain after drought may cause a rise of as much as 20 atmospheres in the suction pressure of roots.

Scott and Priestley (1928), and Scott (1928), considered that the importance of the root hairs as organs for absorbing water from the soil had in the past been over-emphasised. They also considered that the entry of water into the root must be taken into account in relation to the soil moisture. They supposed that when water is present in the soil in excess and is free to move to the plant, the soil solution permeates the walls of the cortical cells, which are composed of cellulose. These cortical cells take up the moisture until they are turgid; apparently acting like a sponge. When the endodermal region is reached, the protoplasts of this layer of cells act as a semi-permeable membrane and the water is drawn in from the outer tangential walls by the osmotic pull of the stelar solution in the inner tangential walls. In these circumstances, it is affirmed that the surface area of the root is of no importance. In drier soils, however, in which the soil moisture is not able to move so easily, the root surface and the root hairs are of importance for the absorption of water, and the latter function as water absorbers. This suggestion is of a somewhat revolutionary nature, and it is unfortunate that the authors, before making such statements, did not bring forward strong experimental evidence in support of such a view. It might be suggested that the soil would have to be water-logged to a marked extent before their first assumption would hold, and water-logged soils are not suitable for the normal growth of plants. This work also takes no account of the complex relationship that is known to exist between the soil and its moisture, a relationship that has not yet been clearly elucidated.

The Soil Solution

The water surrounding the soil particles contains dissolved mineral salts in minute amounts, and it is these mineral ingredients that form the chief source of inorganic food for autotrophic plants as well as the large microflora of the soil.

The isolation of this soil solution has always presented difficulties to the soil investigator. The water extract of the soil has not quite the same composition as the soil solution, although it is not very different. One of the chief ways of isolating the soil solution is by the displacement method of Schloesing. In this method about 30 to 35 kg. of soil are placed in a large inverted graduated bell jar, and water tinged with carmine is poured through. The addition of the water displaces the soil solution, and as the latter descends, it is collected.

More recently other methods have come into vogue. Burd and Martin (1923) employed a battery of soil filtration tubes with air pressure attached. The tubes were made of brass, approximately 3 inches in diameter and 17 inches long. A brass screen was dropped into the bottom of the tube. The screen was covered with filter paper of equal diameter. The tubes were now packed with soil to a height of 10 inches (this approximated to about 2 kg. of moist soil), and 400 c.c. of water was added. The solution that passed through was collected in 10 c.c. fractions. Tests by the electrical conductivity method showed that the first fraction was uniform in composition with the soil solution. Hoagland (1918) has employed the freezing method of Bouyoucos and McCool to isolate the soil solution.

The older investigators held that the soil solution was approximately constant in composition and was made up of a saturated solution of inorganic salts. This, however, is not the case. One of the most important factors influencing the concentration of the soil solution is the soil moisture. Seasonal variations also play their part. Hoagland (1918) found very considerable seasonal variations to occur, and that the concentrations of the soil solution were always uniformly lower in cropped soil than in the same soils uncropped. Under conditions favourable to plant growth, the soil solution was found to be very dilute, particularly at the

height of the growing season. (See also Burd and Martin, 1924.)

Wheeting (1925) has studied the relationship between the moisture in the soil and a salt added to it. He found that there were no salt movements in soils only containing hygroscopic moisture. Increase in the moisture content increased the rate and amount of salt translocation up to a certain point, after which further addition of water showed no increasing effects. In a medium sand, for example, the point of the maximum salt movement occurred at a moisture content of 3 per cent., while the value for silt loam was higher, approximately 10 per cent. He was able to show that there was an important and marked movement of soil moisture, as vapour, towards a salt-treated soil. If capillary connection were cut off there was an accumulation of water round the salt. Treatment of the soil with lime-water brought about a more rapid distribution of potassium chloride and sodium nitrate in both heavy and light soils. Calcium monophosphate, however, appeared to be fixed, and showed no movement. At low temperatures (0° C.) salts moved but slowly in the soil, and every rise in temperature brought about an increased movement.

The rate at which the phosphate concentration of the soil solution is restored after reduction has been much investigated. Von Wrangell (1926) found that in some soils there was a rapid rate of readjustment, while in others the rate was slow. The actual rate was determined by the amount of reserve phosphate in the soil and the absorptive power of the latter. The suggestion is put forward that the phosphate concentration of the liquid close to the surface of the soil particles is greater than in the bulk of the liquid. Thus the root hairs of the plant may be in a position to absorb phosphate from concentrations other than those indicated by analyses of the soil solution.

Parker and Tidmore (1926) have found very similar results to those of von Wrangell. They showed that the addition of lime considerably increased the phosphate content of the soil, after an initial treatment with superphosphate and basic slag. Burd (1925), using the displacement method, has followed the rate at which the concentration of kations is re-established after the original soil

solution had been displaced with water. In soils rich in nitrates the concentration of calcium and other kations was largely determined by the nitrate content, but when this was reduced to a low value by absorption by plants and other means, other anions, especially the bicarbonate anion, took the place of the nitrate. It follows as a corollary that variations in the nitrate concentration of the soil can give no idea of any variations in the kation content.

Parker and Pate (1926) have made a study of the relationship between the saturation of the soil with calcium ions, the hydrogen-ion concentration and the availability of the former. The method employed was to estimate the ease of replacement of the calcium kation using a solution of potassium acetate. They ascertained that the lowest availability always exists in soils with a high hydrogen-ion concentration, and *vice versa*.

Soil Acidity

Soils which give an acid reaction with litmus are termed "acid" soils by agriculturalists. This acid condition was considered to be brought about by the presence of organic acid formed from plant remains under conditions of poor aeration, or perhaps bad drainage or both. Acid soils, however, are also found when both the latter conditions are suitable and the organic matter is low, and in such circumstances the further assumption was made that acidity was due to the presence of inorganic acids, such as aluminosilicic or even silicic acid itself.

The nature of soil acidity has led to considerable controversy, and it is only within recent years that light has been thrown on the question.

The older school of thought explained the phenomenon on the ground of preferential adsorption of basic ions which were considered to be more readily removed than the acidic ions. But the newer trend of work on the clay "molecule" and base exchange in the soil has placed a different complexion on the problem. Whatever may be the cause of acid soils, it has been known to the practical farmer that the addition of lime to the soil will remedy the evil. This is of importance, because acid soils are infertile for

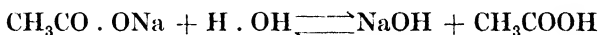
the greater number of crops. Very heavy additions of lime, however, have an injurious effect on crops. Bobko, Goluber and Tulin (1925) considered that this action of lime could not be due to the development of alkalinity in the soil, since on both light and heavy soils the *pH* value was raised to approximately the same figure (8), and the harmful effect of heavy dressings of lime was only shown on light soils. The view is put forward that the toxic action of heavy lime dressings is due to the excessive development of biological activity with the simultaneous development of high concentrations of ammonia, calcium, bicarbonate, nitrate and occasionally nitrite ions. These products attain a higher concentration on light soils owing to their lower adsorptive capacity.

The main difficulty that had always confronted investigators who attempted to explain soil acidity as being due to the presence of acid was the fact that no undeniable acid had been obtained from the soil except in small amounts. But whatever the cause of soil acidity, there is only one method of accurately stating the effective acidity, and that is by using the hydrogen-ion concentration of the soil solution or soil extract. This may be done by giving the hydrogen-ion concentration in grams per litre or by using the expression *pH*, which, as is well known, is defined as the logarithm of the reciprocal of the hydrogen-ion concentration expressed in grams per litre. The hydrogen-ion concentration can be measured by the accurate hydrogen-electrode method or by the very much simpler method of using appropriate indicators. There are, however, certain disadvantages attached to the latter method, such as turbidity of the solution, which makes it difficult to see the exact colour of the indicator. For a full discussion of these difficulties, see Fisher (1921) and Atkins (1924).

A number of methods have been employed to classify and denote the acidity of the soil. Wherry (1919) distinguished the types: miniacid (*pH* 7 to 6), subacid (*pH* 6 to 5), mediacid (*pH* 5 to 4), superacid (*pH* 4 and less), and corresponding terms were used for the alkaline region. Lemmerman and Fresenius (1922) distinguished three types of soil acidity: (1) actual acidity of the soil solution due to the presence of acids; (2) latent acidity, which is developed in the presence of neutral salts owing to the

liberation or basic exchange of easily hydrolysed aluminium and iron salts ; and (3) latent acidity developed as the result of salts of weak acids with strong bases. In such circumstances it was supposed that the base was adsorbed by the soil colloids and the acid left.

Kappen (1924) and his co-workers in Germany (see Kappen and Bollenbeck (1925), Kappen and Beling (1925)) distinguished four types of soil acidity, which they appeared to consider as due to distinct phenomena. When a soil becomes acid owing to lack of lime, the first type of acidity, "hydrolytic acidity," is said to develop. This is explained on the grounds that in only a mildly decalcified condition can the soil take up the base of a hydrolysed salt and leave the free acid in solution. When, for example, sodium acetate solutions are added and the liquid gives an acid reaction, Kappen assumed that the soil adsorbs the hydroxyl ion together with the sodium ion and the equilibrium



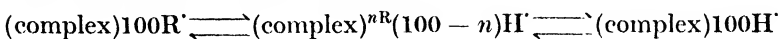
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The second type, of acidity, termed "exchange acidity," which is developed when a neutral salt is added to the soil solution was considered to be due to the direct exchange of aluminium ions with the kation of the neutral salt, the aluminium being present in the soil silicates as kation.

The third type, termed by Kappen "neutral salt decomposition," is again brought about by the addition of neutral salts to the soil solution. But in this case they give rise to a still higher hydrogen-ion concentration, which is not necessarily followed by an equivalent exchange of aluminium ions. This state of affairs is said to be especially applicable to humic soils. Here the acidity was supposed to result from the adsorption of hydroxyl ions which were considered to increase in concentration in the presence of the neutral salt. The hydroxyl ion takes with it the kation of the neutral salt, and this results in an increase of the hydrogen-ion concentration of the solution.

The last type of acidity, "active acidity," develops in a soil completely depleted of exchangeable bases. Kappen and his fellow-

workers entirely rejected the theory of Gedroiz (1924) and Hissink and van der Spek (1925), who regarded hydrogen as an exchangeable kation and explained soil acidity phenomena on this ground. Bradfield's investigations (see above) showed very clearly why the colloidal matter of the soil should be regarded as being truly acidic in nature, and since this colloidal acid is in the gel state surrounding the surface of the particles, ordinary stoichiometric formulæ cannot be applied to the system of aluminosilicic acids and their associated kations. But if n per cent. of the total saturation capacity of the colloidal acid be neutralised by basic kations, this may be written $(\text{complex})^{nR}(100 - n)\text{H}'$, and the whole range between complete saturation and complete desaturation will be given by the expression



the left-hand formula $(\text{complex})100\text{R}'$ will correspond to the normal salt, the right-hand $(\text{complex})100\text{H}'$ will represent the free acid and the intermediate formula $(\text{complex})^{nR}(100 - n)\text{H}'$ the wide range of acid salts found in the soil. It is a well-known fact that complete saturation corresponds to a $p\text{H}$ of 10 or 11, whereas complete desaturation corresponds to a $p\text{H}$ of 3.5 to 4. The greater the value of n , the greater will be the value of the $p\text{H}$, *i.e.*, the greater the alkalinity of the soil. Hissink's results show that when R represents calcium, neutrality ($p\text{H} = 7$) corresponds to a value of $n = 5.5$ for a number of soils. Kappen's soil acidity classification can be better explained in terms of the variations exhibited by n in the formulæ given above. The higher the value of n , the lower will be the percentage of hydrogen ions in the complex, which are capable of exchange with other kations, thus lowering the equilibrium concentration of hydrogen ions in the liquid when the soil is treated with a soluble salt. In a soil with a comparatively small proportion of acidic hydrogen in the colloidal phase the salt of a strong acid, such as hydrochloric or sulphuric acid, can only bring about a minimal amount of ionic exchange between the kation and this ionic hydrogen, since a low quantity of titratable acid in the form of hydrochloric acid will be sufficient to effect the small equilibrium concentration of hydrogen

ions in the liquid. In the case of the sodium salt of a weak acid, *e.g.*, sodium acetate, a greater amount of acidic hydrogen can be exchanged for sodium before the same hydrogen-ion concentration is reached in the liquid, for this concentration represents a much higher titratable acidity for a weak acid. It is only in highly desaturated soils, in which the value of n is lower, that the amount of acidic hydrogen present in the colloidal complex is sufficiently high for ionic exchange with the salt of a strong acid to give appreciable amounts of titratable acidity.

It will be seen from these results that soils contain two groups of acids, inorganic acids of the clay complex, such as aluminosilicic acid, and organic acids, such as humic acid. In soils with a pH of 7, *i.e.*, neutral soils, both the organic and inorganic acids are chiefly in the form of their calcium salts, together with other bases and hydrogen ions. In acid soils, on the other hand (pH less than 7), more hydrogen ions are replaced with the result that hydrogen ions preponderate over hydroxyl ions in the soil solution. As Russell remarks, "Acid soils are characterised by three properties—deficiency of replaceable calcium, presence of hydrogen ions, and a tendency to contain soluble aluminium and iron salts."

Soil Reaction in Relation to Plant Growth and Distribution

The acidity of the soil may in itself be injurious if it be sufficiently great, or it may simply mean that the soil is deficient in calcium salts, and it is to their absence that the infertile condition of the soil is really due. Or again, acidity may be due to the presence of toxic aluminium ions. The reaction of the soil has undoubtedly a very profound effect on the growth of soil bacteria, protozoa, fungi and algæ, and the problem has been critically investigated by several workers from this standpoint.

Gillespie and Hurst (1918) found that potatoes grown in a soil of pH 5.2 or less were rarely affected by scab. For example, on Caribon loam (pH 4.8) no infection of the tubers occurred, whereas if the plants were grown on Washington loam, which has a higher pH , there was bad infection of the tubers. They drew the important conclusion from this investigation that absolute

neutrality of the soil is not necessarily the most suitable reaction for the growth of a given crop. Similarly Martin (1920) discovered that *Actinomyces chromogenus*, the cause of potato scab, may be checked in Irish cobbler potatoes by increasing the acidity of the soil. In a sample of soil with a pH of 6.03 at the beginning of the experiment, he found that the addition of sulphur at the rate of 400 to 600 lb. per acre altered the pH to 5.2 and 5.07 respectively. With this treatment there was a decrease in unsaleable potatoes from 42.9 per cent. in the treated soil compared to 8.9 and 7.5 per cent. in the untreated soil, although the total crops were approximately the same in both cases.

Some plants appear to have a narrow range of pH suitable for growth. Good examples of such cases are to be found in the Orchidaceæ, Ericaceæ and Leguminosæ. It is possible that in the first two families the presence of mycorrhiza in the roots may have something to do with the problem, while in the Leguminosæ the bacteria in the root nodules are sensitive in their distribution to the pH values of the soil.

Hartwell and Pember (1918) have found that acid soils affect barley and rye differently, due apparently to the fact that aluminium salts are highly poisonous for barley, but exert no action on rye. Blair and Prince (1920) have made measurements of soil pH in Western Virginia, and they found that the optimum pH values were obtained when the phosphoric acid and sodium hydrate were the adjusting substances. Seedlings of soya-bean and lucerne gave optimum growth in a soil of pH 5.94, whereas maize required a slightly increased acidity (pH 5.16). In more strongly acid soils, soya-beans and wheat suffered little until the values fell below 5.16. Lucerne suffered at once if there was a reduction in pH . A pH of 2.96 seemed to be below the critical value for growth and 2.16 proved to be fatal, although germination occurred at this high acidity and the growth of moulds was strong in such an acid medium. A certain amount of injury was observed when the neutral point was reached and considerable injury was wrought when the neutral point was passed and the alkaline region reached. Thus alkalinity is more injurious to these plants than acidity. Salter and McIlvaine (1920), working in New Jersey and using

sulphuric acid and calcium carbonate for adjusting the *pH*, found that the germination of lucerne was practically unaffected between a *pH* range of 4.5 to 7.0, while below the former value it was much inhibited. The yield showed a steady increase between *pH* 3.8 to 6.5, and irregularities occurred between 6.5 and 8.

Haas (1920) has attempted to analyse the way in which acidity injures a plant. The sap of buckwheat seedlings showed a *pH* of 5.48 to 5.97. These figures may be of the same order as the optimum condition in the soil. Some of the results lend colour to the suggestion. Truog (1920), for example, found that the harmful effect of soil acidity exceeding these values was due to its influence in preventing plants securing rapidly enough the bases necessary for neutralising and precipitating acids within the plants, and, in general, addition of lime was discovered to decrease the acidity of the plant sap.

Crops vary widely in their sensitivity to soil acidity. Olsen (1925) showed that although the optimum range of *pH* for lucerne (6.5 to 7) differed little from that for rye (6.0 to 6.5) or buckwheat (6.0 to 7), the lucerne gave 13.0 per cent. of optimum growth in a soil of *pH* 4.0, whereas buckwheat and rye at this reaction gave 90 and 82 per cent. respectively of the growth at the optimum *pH*. Olsen's work, as well as that of Trénel (1925), showed that the majority of plants favour a slightly acid or neutral soil reaction, and that excess of alkalinity has a far greater depressing effect than excessive acidity.

Arrhenius (1925) has investigated the growth of common farm crops in soils of varying reaction, and has claimed to have shown that the curves illustrating relationships between plant growth and *pH* of the soil invariably have two maxima. Olsen, however, failed to confirm this statement. Arrhenius did not find infertility of soils due to the presence of aluminium ions as a phenomenon of frequent occurrence, since toxic concentrations of this element are only found in very acid soils.

A good deal of attention has been focussed within recent years on the presence of soluble aluminium compounds in acid soils. Hardy (1926) has published a critical review of this question, and the following account is a condensed report of his paper.

When acid soils are extracted with solutions of neutral salts, considerable amounts of aluminium, as well as iron and manganese, are found in the extract. Aluminium in acid soils was regarded as a direct cause of their infertility, and soil acidity came to be looked upon as mere secondary consequences of the known susceptibility of aluminium salts to hydrolysis. Subsequent work showed that aluminium could be detected by ionic reactions in soil extracts only when their hydrogen-ion concentration was higher than that of pH 4.5. It was therefore next assumed that in less acid soils and soil extracts the soluble aluminium was present as an unionised hydrated alumina hydrosol. The fact that aluminium in acid soils and extracts was found to be unable to pass through semi-permeable membranes was considered to be strong evidence in support of such a view. It then came to be maintained that so far from soil acidity being a consequence of the hydrolysis of aluminium salts, the reverse was the case. Ionised aluminium was supposed to occur only when the acidity of the soil or soil extract was sufficient to convert the ionised hydroxide into the ionised salt. The recent work of Magistad (1925) has also considerably added to our knowledge in this respect. He studied (1) the influence of the hydrogen-ion concentration on the solubility of aluminium originally present as sulphate, (2) the amount of aluminium in soil solutions displaced from soils of various reactions, and (3) the particular form in which aluminium occurred in soil solutions. He showed that between the pH limits of 4.7 and approximately 8, only small amounts of aluminium existed in solution, either in simple aqueous solutions derived from aluminium sulphate or in displaced soil solutions, and that these small amounts existed in colloidal dispersion and not in true ionic or molecular solution. He therefore came to the conclusion that aluminium should not exert poisonous effects on plants unless the reaction of the medium lay outside the range of pH 4.7 to 8.5.

Hardy also discussed the position in regard to recent investigations of physico-chemical properties of hydrated alumina with special reference to the iso-electric point, the formation of co-ordinated complex anions and organo-compounds of soluble alumina in both these forms, as well as in the form of simple ions

or more complex colloidal electrolytes. These may possibly enter the root cells and be translocated within the plant, but they appear to exert true toxic effects only when in the form of simple ions or more soluble colloidal electrolytes. Thus the absorption of aluminium in other non-poisonous forms may result in the accumulation of aluminium in certain regions of the plant body, with consequent disturbance of its metabolic processes, and predispose it to certain diseases. The use of dialysis experiments, upon which the above views have been founded, are, however, of doubtful value, since dialysis itself disturbs markedly the equilibrium conditions in hydrated colloidal systems, and in any case the membranes of dialysers do not resemble the membranes of plant cells. Nevertheless, there is a considerable volume of evidence from other sources in support of the conclusion that within the reaction range at which poisonous aluminium compounds can have no existence in soils (a range which includes that of the reaction of most normal soils) hydrogen ions may exhibit and exert a controlling influence on plant growth. It is, however, not admissible in this connection to apply a strict reaction range to all soils. Moreover, the variations in the sensitiveness of different species of plants to the reaction of the soil must also be taken into account.

Salisbury (1921, 1925) has pointed out that in the relation of pH to plant distribution, under the protected conditions of artificial cultivation, species can tolerate conditions of acidity to a degree not found in nature. "Because aquatics such as *Butomus umbellatus* or *Alisma plantago* will grow and flower in ordinary garden soil, or *Statice binervosa* on stiff clay, apparently more vigorously than on its native shingle, no one would suggest that the former are not rightly regarded as aquatics or that the latter is not really a littoral plant, finding in these respective natural habitats the optimum conditions for establishment and maintenance under natural conditions of competition."

Within fairly narrow limits, owing to its strong buffering action, the acidity of a soil left undisturbed is fairly constant throughout the year. Salisbury investigated natural woodlands in relation to hydrogen-ion concentration and plant succession,

Owing to leaching action, the surface soil was found to be poorest in bases, which increased in amount with increase in depth. On the other hand, the organic content decreased rapidly with depth, and a gradient of hydrogen-ion concentration was present with a maximum value at the surface. The presence of living organisms on the surface layers of a woodland soil provide such a volume of carbon dioxide as naturally to increase the proportion of this gas in the atmosphere at ground level. Thus the rain, as it percolates through the carbon dioxide-laden interstices of the soil, becomes charged with carbonic acid and forms an efficient leaching agent for the underlying layers of soil. The insoluble calcium carbonate thus formed from the calcium present in the soil becomes converted into the soluble bicarbonate. It was discovered that in many *Quercus sessiliflora* woods, as well as in those of other types, the surface layers of the soils might be quite devoid of calcium. In the case of light soils, where percolation is more free to take place and the clay fraction is small, complete leaching could take place to considerable depths. Salisbury considered that an important factor which militates against excessive leaching is the fact that deep-rooted trees and shrubs are constantly removing mineral salts from unleached lower layers of the soil so long as they are alive. But certain elements, especially calcium, are present in considerable amount in dead leaves, and soil adjustment from this source varies very considerably according to the species. The greatest amount was found in the case of the beech (2.46 per cent.) and the least in heather (0.44 per cent.). A soil with a high organic content exerts a powerful buffer action which decreases with the decrease in organic matter. Salisbury advanced the view that woodlands generally and probably all types of undisturbed plant communities in this country are tending to become progressively more acid in character, with consequent change in the nature of the vegetation.

Calluna vulgaris is a plant which is said to prefer acid conditions and is not tolerant of chalk soils. According to Hinchcliffe and Priestley (1924), plants growing on badly aerated peat moors characteristically produce large amounts of fatty substances in the form of fat deposits, such as thick cuticles. The suggestion is

therefore made that the presence of calcium would tend to form insoluble soaps with these fats and so choke the tissues behind the growing point, and it is on this account that calcium is not tolerated by these plants. It is difficult to see how such an explanation can possibly fit the facts of the case, and, moreover, it is beyond rigid experimental proof.

The problem of reducing soil acidity has been studied by Robinson and Bullis (1921) in some acid soils of Oregon. Lime was added to the soils, but they did not respond to this treatment, and at present no explanation is forthcoming to explain this curious result. It is a well-known fact, however, that some infertile soils are greatly benefited by the addition of lime. Such soils are spoken of by farmers as "sour" soils. Curiously enough, as a general rule, sour soils do not have a low pH , so that soil sourness and soil acidity are not necessarily synonymous terms. The absence of calcium is probably the chief cause of sour soils. Pearsall (1926) pointed out that excessive leaching is one of the principal causes of soil sourness, and that this leads to an increase in the ratio $\frac{Na + K}{Ca}$ in the soil. He attached great importance to this ratio in this connection, but it is very doubtful if it is really as all important as he attempts to argue.

Aeration

Adequate aeration of the soil is necessary for the normal growth of plants. Certain plants, however, can grow in situations in which the supply of oxygen to the roots is limited. Aquatics in still water and bog and marsh plants can grow in badly aerated conditions. As a general rule, the chief anatomical feature of such plants is an intense development of air spaces in the cortical region of the root.

Two completely different factors are concerned in this question of aeration : (1) an adequate supply of oxygen, and (2) the harmful influence of the carbon dioxide formed from the respiration of roots. In badly aerated soils or culture solutions this gas tends to accumulate, and exerts a toxic action on the well-being of the plant.

The reactions of plants to aeration of the root system are variable. Pember stated that daily aeration of solutions in which barley was growing did not noticeably change the growth of the plants. This result can be explained by the fact that the solutions were changed every fourteen days, and water was frequently added to make up for water loss. In fact, it was shown by Free in 1917, two years previous to this work, that barley is particularly sensitive to poor aeration and lack of oxygen, but buckwheat is not; a result that has been confirmed by Stiles and Jørgensen (1917). Bergman (1920), in a comprehensive study of the behaviour of various plants, found, on growing the seedlings of beans, balsam and geranium in pots submerged in bog or tap water until the top of the soil was covered, that the balsam wilted in two days, was severely wilted in three, and beyond recovery in four days. The geranium and bean seedlings took longer to show wilting (four to five days), and at the end of six days the leaves had turned yellow and dropped. Under similar conditions *Cyperus* and *Ranunculus* grew vigorously. In a second series of experiments air or oxygen was supplied to the plants as soon as they showed signs of wilting, with the result that they regained their turgor, provided they were not too wilted, and grew normally for as long as aeration was continued. In eight to ten days all the plants developed new roots at or near the surface of the water, after which aeration could be discontinued. Bergman (1921) has also studied the relation between oxygen-content and the injury of the cranberry vine due to flooding. It was discovered that injury was most apt to occur during cloudy weather, when the oxygen supply was lowest. When submerged cranberry vines were shaded, injury resulted from the reduction of oxygen concentration of the water. No essential difference was found when the shaded vines were in pond or bog water. The flowers and growing tips were the parts most affected, owing possibly to their higher rate of respiration and consequent greater demand on oxygen supply. Knight (1924) grew maize, wallflower and *Chenopodium album* in soil and culture solutions. He found that there was an increase in the dry weight of the maize when the soil was aerated, but aeration produced no increase when the plants

were grown in culture solution. With the wallflower and *Chenopodium album* aeration of both soil and culture solutions led to an increase in dry weight.

The atmosphere of the soil has been much investigated, and is of importance in its bearing on making the soil a suitable medium for plant growth. The amount of carbon dioxide increases with the depth of the soil, and the oxygen correspondingly decreases. Seasonal changes have a marked effect upon the composition of the soil atmosphere. The carbon dioxide content varies with the time of year. There is always a higher concentration in the summer than in the winter. The organic content of the soil also influences the carbon dioxide concentration of the soil atmosphere, and temperature and moisture play a part as well. Sand contains the least carbon dioxide and the most oxygen, while calcareous sand and clay have about double the concentration of carbon dioxide, and moor soil is the richest in this gas. The addition of organic matter tends to enrich the carbon dioxide concentration of the soil at the expense of the oxygen. The vast soil flora and fauna also have an important rôle in increasing the carbon dioxide of the soil. The porosity of the soil is of direct use in this connection and determines the rate at which oxygen can enter and carbon dioxide escape. The water-logging of soils is practically confined to soils of heavy nature. Such soils only contain oxygen in solution, and the amount is not sufficient for land plants, which do not possess the aerating system of aquatics, and as a result the former die from want of oxygen.

The amount of carbon dioxide which proves to be toxic to plants varies with the species. The range may be anything between 2 and 20 per cent. As a matter of fact, such concentrations are by no means rare in the soil, and carbon dioxide injury to roots is a more frequent phenomenon than is generally supposed, especially in field and garden soils that have been heavily manured.

Livingston and Free (1917) found that absorption by the roots of *Coleus blumei* and *Heliotropium peruvianum* ceased within twenty-four hours after replacing the soil air with nitrogen. In one to six days this was followed by general loss of turgor in the shoot and leaves, and was finally followed by wilting and death.

In *Nerium Oleander* the disturbance of the water relations in the shoot was indicated by yellowing and loss of turgor in the leaves. *Coleus blumei* recovered slowly when oxygen was once more admitted, but *Heliotropium peruvianum* failed to do so after wilting had become extensive. The roots of the injured plants were found to be dead and partially disintegrated. New roots were formed from the base of the stem in *C. blumei* on the admission of oxygen. The injury due to lack of oxygen was found to be greatest in plants with large root systems, yet *Salix* was found to function normally in the absence of oxygen. Bergman (1920) observed that geranium plants wilted in a few days after the roots were subjected to carbon dioxide, and it was found that the wilting commenced before all the oxygen had been replaced. After wilting, the leaves turned yellow and fell off towards the end of the experiment. *Impatiens balsamina* under similar treatment was slightly wilted on the second day and badly wilted on the third. When given access to air once more it failed to recover.

Cannon (1916) determined that exposure of the roots of *Prosopis* and *Opuntia* to pure carbon dioxide for fifteen minutes did not affect growth, but exposure for periods exceeding thirty minutes produced harmful effects on growth. He also found that root growth regularly ceased in nitrogen, though in some cases it might continue for a day or two. The effect upon the shoot was much less marked; in *Eriogonum*, growth continued at the rate of 8 to 15 mm. per day for eight days after the roots had been subjected to nitrogen. Shoots of the potato, on the contrary, ceased growth almost immediately after the roots had been submitted to an atmosphere of nitrogen. Cannon also found that carbon dioxide in concentrations of 25, 50 and 75 per cent. exerted a depressing effect upon the root growth of *Krameria canescens* and growth that came to a standstill after a time, but was renewed on the admission of oxygen. In *Covillea* the effects of large concentrations of carbon dioxide proved to be very harmful, and growth was quickly inhibited. In the succulent *Mesembryanthemum* growth was depressed when the roots were treated with large concentrations of carbon dioxide, but it only completely ceased

after relatively long exposures, and was renewed with relative rapidity on the readmission of oxygen. Thus the difference in sensitivity of these three species to carbon dioxide is shown by the fact that the root growth of *Covillea* ceased in 1·5 hours in 25 per cent. of carbon dioxide, that of *Krameria* in 2·5 hours, and *Mesembryanthemum* in over four hours. In further experiments with such low concentration of oxygen as 1 to 2 per cent. in nitrogen, Cannon again demonstrated the variability of species in their reactions to lack of oxygen. When the concentration of oxygen was reduced to 2 per cent., growth ceased immediately in the onion and was much retarded in most other species that were used. In plants inhabiting wet soils, however, such as *Juncus* *Salix*, *Potentilla* and alfalfa growth was but slightly affected.

Cannon and Free (1919) have shown that the root growth of the sweet pea stopped at once in static atmospheres of nitrogen and helium, but continued for three days in a stream of helium at a rate but slightly below that of normal air. Similarly, injury to the roots of potatoes took place very much more quickly in nitrogen than helium. In a later series of experiments (1920) these workers showed that this difference in action between helium and nitrogen does not occur in completely anaerobic conditions or when an ample supply of oxygen is present. Thus with nitrogen, 1·5 per cent. of oxygen is required for the root growth of the sweet pea, but with helium only 0·5 per cent. is required; a considerable difference. The suggestion is made that the difference exhibited by these two gases is due to the fact that oxygen diffuses more rapidly through helium than it does through nitrogen.

As far back as 1907, Clements recognised that the air content of the soil atmosphere was an important factor in all soils, especially in acid ones, and the view that sufficient aeration is a prime necessity for successful plant growth has gradually come to the fore ever since. Balls (1912), for example, showed that the roots of the cotton plant were locally asphyxiated in water-logged soils, and death and decomposition followed in a few weeks. Harrison and Aiyer (1913) reached a similar conclusion in India for the rice crop. The aeration of the forest soils of India was investigated by Howard and Singh (1914) and Howard and

Howard (1918 to 1920), their work having had practical application in the indigo crop. It was shown by Howard and Howard that indigo wilt was due to insufficient aeration of the soil. If floods cause the ground water to rise, or if heavy rainfall waterlogs the surface soil for long periods, the defective aeration which results from these conditions makes root regeneration difficult and wilt ensues. In confirmation of this view it has been shown that other deep-rooted species exhibit wilt under such conditions, whereas shallow-rooted species do not. Moreover, wilt is common in years of heavy rainfall and rare and of slight importance in dry years, and lastly it is seldom found on porous soils.

Clements (1920) has recognised the fact that plants may serve as indicators of good or bad aeration of the soil, and considered that the presence of acids and toxins in soils is a direct result of bad aeration.

The Root System of Plants

It is unfortunate that the root system of plants has not received the attention of the aerial portions at the hands of the physiologist. This is no doubt due to the fact that it lies underground, and is therefore of difficult accessibility and at the same time does not lend itself very readily to experimental treatment. Certainly this highly important region is of great physiological importance, for it is the moisture- and salt-absorbing region of the normal green plant, and at the same time acts as an anchorage for the plant to its substratum.

An important ecological and physiological investigation of different root systems has been conducted by Weaver (1919, 1920), and the following is mainly a summary of this work. The method employed was to dig trenches 2 to 3 feet wide and 6 to 10 feet long to a depth of about 6 feet by the side of the plant to be examined. This offered an open face on which to work, and by careful digging with a hand-pick the root system could with practice be removed in its entirety. As the work proceeded the trench was deepened, often to a depth of 20 feet or more. The root systems were removed and photographed or sketched on an exact scale.

In the prairies of Nebraska some thirty-one perennial plants

were investigated. *Panicum virgatum*, although it showed a preference for loose sandy soils, grew abundantly in many positions throughout the prairies. The roots of this plant are very coarse and the longest of any grass that was investigated. Several reached a vertical depth of 8.5 feet. In the first 6 or 7 feet there was little branching and laterals only occurred sparingly. In *Andropogon furcatus* there is an abundant root system which grows vertically and obliquely downward and forms a dense sod. The greatest depth reached by the roots is 6 feet 10 inches, and all the roots show profuse branching. *A. scoparius* extends to a depth of about 5 feet in sandy soil. In gravel soil mixed with sand and a rocky subsoil of decayed sandstone the roots only reached to a depth of 3 feet. With clay loam soil and clay subsoil the length of the roots only extended to 28 inches, whereas in clay loam alone the roots extended to 65 inches. *A. nutans* is a deep-rooted form. The maximum depth of root is found to vary between 51 and 59 inches. There is very little lateral expansion, and the roots completely occupy the soil by profuse branching to the second and third order. Other grasses investigated were *Stipa spartea* (21 to 26 inches), *Koeleria cristata* (15 inches), *Elymus canadensis* (16 to 22 inches), *Agropyrum repens* (2 to 3 feet), *Distichlis spicata* (18 inches to 2 feet), an important inhabitant of alkaline soils which is of considerable forage value, *Sporobolus longifolius* (17 to 40 inches), which occupies a wide area and the roots of which are very dense.

Of the other perennials investigated, *Solidago rigida* possesses a root system which spreads immediately below the soil surface to a depth of 12 to 18 inches on either side of the plant. The roots are very abundant for the first 2 feet of the soil, and the maximum depth that was ever found was 5 feet. *Solidago canadensis* behaves in the same way as *S. rigida* and branches freely below the surface, but often reaches as great a depth as 9 to 10 feet.

The most obvious conclusion to be drawn from a consideration of these data is the fact that prairie plants are provided with a well-developed, deep-seated and extensive root system. Weaver divided the thirty-three species that were examined upon a basis of root depth into three classes :—

(I.) Shallow-rooted plants not extending below the first 2 feet of soil. This class consists wholly of grasses, such as *Koeleria cristata*, *Stipa spartea*, *Elymus canadensis*, *Distichlis spicata*, *Sporobolus longifolius*, and *Aristida oligantha*.

(II.) Plants with roots extending well below the second foot of soil, but seldom deeper than 5 feet. Here belong: *Andropogon scoparius*, *A. nutans*, *Bouteloua gracilis*, *Bulbilis dactyloides*, *Verbena stricta*, *Helianthus rigidus*, *Solidago rigida*, *Petalostemon candidus*.

(III.) Of the plants placed in this section, 55 per cent. have roots which extend beyond a depth of 5 feet; indeed, most of them to depths of 7 to 9 feet and a maximum depth of from 13 to 20 feet or more. They may therefore all be classed together as deep-rooted species. The following are placed in this class: *Panicum virgatum*, *Andropogon furcatus*, *Agropyrum repens*, *Solidago canadensis*, *Astragalus crassicaarpus*, *Psoralea tenuiflora*, *Lygodesmia juncea*, *Ceanothus ovatus*, *Baptisia bracteata*, *Lespedeza capitata*, *Glycyrrhiza lepidola*, *Brauneria pallida*, *Vernonia baldwinii*, and *Kuhnia glutinosa* (Fig. 4).

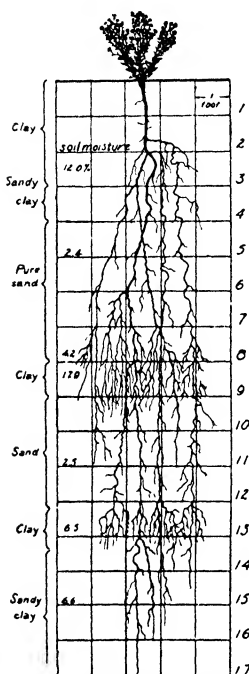


FIG. 4.—The root system of *Kuhnia glutinosa*, showing the deep penetration in prairie soil. (After Weaver, *Ecological Relations of Roots*.)

live under semi-arid climatic conditions in which water supply is the chief limiting factor for growth. During certain portions of the growing season extreme xerophytic conditions are brought to bear on the vegetation. The water content of the soil is brought to the non-available point as far as a depth of 4 to 5 feet in certain years, and especially when the evaporating power

of the air is high. It is in response to these environmental conditions that many species have developed extensive root systems. For just as the evaporating power of the air and the nature of the transpiring organs determine the water loss, so the soil moisture and the nature of the root systems determine the supply. Thus great root depth is to be correlated with deep soil moisture. The nature of the soil also has a marked effect upon root development. Several species, including grasses, penetrate to a depth of 2 to 3 feet in loamy soil, but to a lesser depth in the same type of soil when it is underlaid with a hard subsoil of clay.

The chaparral community lying between the Ohio-Missouri deciduous forest complex and the prairies to the westward was also investigated. Here *Symphoricarpus*, *Rhus*, *Corylus* and *Rosa* play the part of dominants. *Symphoricarpus vulgaris* forms dense shady clumps which exclude even the tolerant *Poa pratensis*. The maximum root depth reached by this plant was 65 inches, but the lack of linear extension is made up by a remarkably well-developed absorbing surface. *Rhus glabra* is never found to extend to a depth of more than 80 inches. In *Corylus americana* the roots extend to great depths, 10 to 11 feet, and in *Rosa arkansana* the tap root reaches the greatest depth of all these species, and grows vertically downward for 21 feet 2 inches.

It will be seen that all the members of the chaparral community possess well-developed absorbing systems, which, though showing variability in their length of vertical extension, are always deep-seated. They are all well adapted either by means of above-ground or under-ground rhizomes or root offshoots to invade effectively, if slowly, prairie sod. *Symphoricarpus* extends its area by above-ground stems as well as by those below the surface. The above-ground stems furnish the more rapid method of migration, but they frequently fail utterly to become rooted in prairie sod. The under-ground rhizomes, however, supply a more certain method of establishment. Once this shrub has established itself, it quickly reacts on the habitat by modifying the composition of the soil and increasing the water content of the

air. The presence of shrubs decreases wind movement, and their shade reduces light and temperature. These factors help to decrease the evaporation of water from the soil surface and conserve the moisture of the soil. The run-off is greatly reduced as a

result of the rich mulch of fallen leaves and large quantities of wind-transported *débris* of plants which become lodged among the stems. Drifts of snow also find lodgement between these shrubs, and when they melt, add considerably to the soil moisture. In a few years from the addition of vegetable matter to the soil, considerable quantities of humus fill the former prairie soil. *Rhus* has very similar effects upon the habitat to *Symphoricarpos*. But since it is a taller shrub its action on the bordering grassland is more pronounced.

The prairies of south-eastern Washington and adjacent Idaho represent an extreme western extension of the great grassland formation lying east of the Rocky Mountains. Here *Agropyrum spicatum*, *Festuca ovina ingrata*, *Koeleria cristata* and *Poa sandbergii* are dominants. *Stipa* and late blooming grasses are absent.

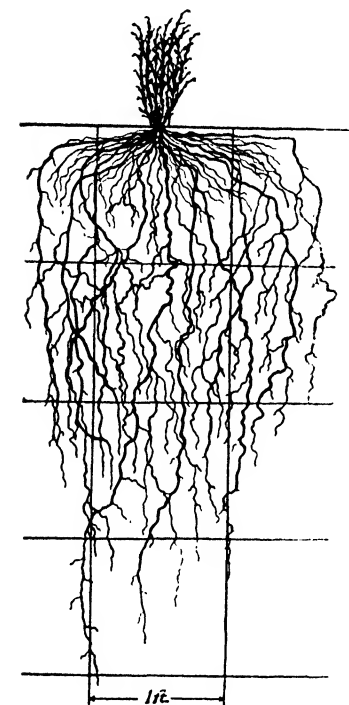


FIG. 5.—*Artemisia frigida*; an example of a root system of the plains association. (After Weaver, *Ecological Relations of Roots*.)

The distribution of rainfall probably accounts for the latter fact. During the whole growing season the rainfall is 5 inches, and except for the highly retentive loam-silt soil, the region is practically desert. The maximum depth of penetration of roots is shown by *Agropyrum spicatum* (4 feet 10 inches), while the other dominants, *K. cristata*, *P. sandbergii* and *F. ovina ingrata*, are all

shallow-rooted plants, and the bulk of their absorbing system lies above the 18-inch level.

The root system of plants living on the plains also shows a deep penetration. In only a few cases do they have an extension of less than 2 feet into the soil. Nevertheless, they are not found to reach the depths attained by the prairie flora. Most of the species dwelling on plains show, in addition to their deep-rooting system, a fine system of roots near the surface with wide-spreading laterals (Fig. 5).

The annual rainfall in such regions is generally small (15 inches), and the major portion of this precipitation falls during the growing season. Such a seasonal distribution of rainfall is very favourable for the growth of grasses. The soil moisture of the plains is not high, and in some cases it was found that the soil may be uniformly dry for as great a depth as 7 feet. It is possible that it is to these factors that the deep root penetration of plants inhabiting plains is due.

The climatic factors of the sandhills area near Colorado are very similar to those of the plains. The root system is not deeply developed. Eight of the nineteen species investigated had roots which were almost entirely confined to the first 2 feet of soil. Even the deep-rooted forms, such as *Eriogonum microthecum* and *Artemisia filifolia*, show an excellent development of laterals in the surface layer. In the soft substratum of the sandhills such a marked development of lateral root growth cannot be attributed to mechanical hindrance to penetration, but is probably connected with the water supply. Any rain is immediately absorbed and there is no run-off. As soon as the rain has ceased, evaporation dries the surface soil with great rapidity, but evaporation does not proceed to any great depth. The surface layer of dry soil thus formed acts as a mulch and retards any further evaporation. At a depth of a few inches below the surface the sand is always found to be moist, and can even be moulded into lumps with the hand. Covering vegetation is also scarce and loss of water from transpiration correspondingly small (Fig. 6).

The root system of plants growing at an altitude of 8,000 feet in the Pike's Peak region of the Rocky Mountains was also in-

vestigated. Here the soil is composed of disintegrated granite, and the degree of disintegration and decomposition largely determines the type of plant community occupying any particular area. All the plants inhabiting this area possess roots well adapted to secure moisture and nutrients from the surface area of the soil. The roots are characterised by a shallow widely spreading system practically confined to the first 18 inches of the soil and showing the best development in the first 12 inches. The lack of depth

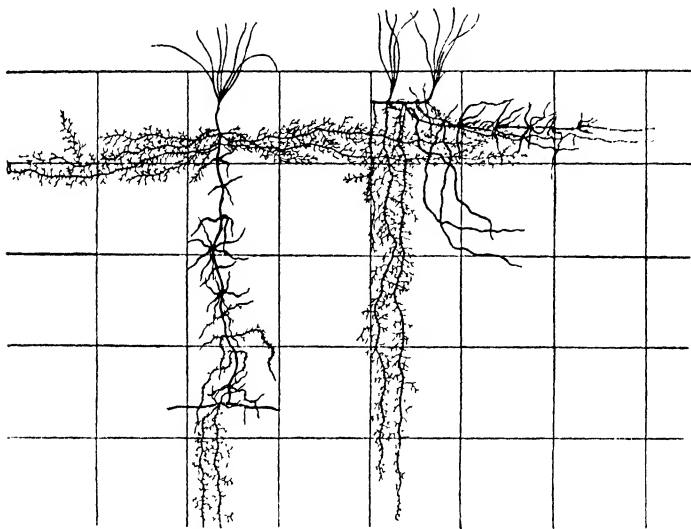


FIG. 6.—*Redfieldia flexuosa*, showing the distribution of rhizomes and roots in the sandhills subclimax association. (After Weaver, *Ecological Relations of Roots*.)

extension is compensated by lateral extension and profuse branching. These adaptations find a ready explanation from the nature of the soil and the distribution of rainfall.

The soils of the gravel-slide consist of a superficial layer composed of coarse angular rock particles which vary in size from fragments of over an inch to a few millimetres in diameter. Except during periods of rain, the surface is very dry, and as the slope is steep, there is often a constant movement of rock particles down the mountain side. Most of the plant tops were found to

have slipped down the slope from 2 to 8 inches or more. This surface layer is very efficient in preventing any run-off, and at the same time forms a dry mulch which protects the soil from excessive evaporation. It was found that the soil moisture was at no time very great, but was evenly distributed throughout the first 18 inches of soil. The evaporating power of the air in this region is high, no doubt partly due to the considerable air movements of such a high altitude. The root system is clearly a response to

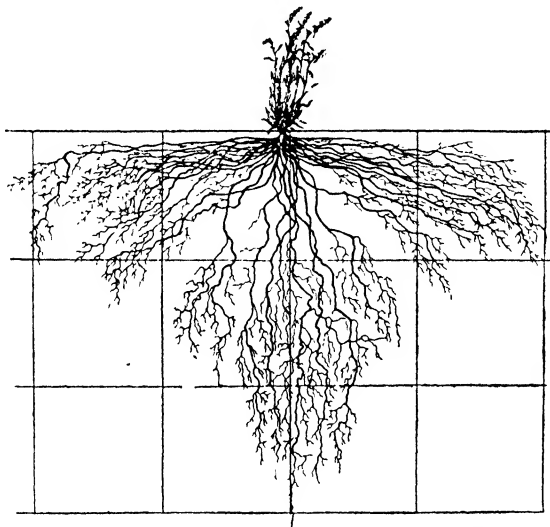


FIG. 7.—*Solidago oreophila*, a plant of the half-gravel-slide community, showing wide-spreading lateral and deep central roots. (After Weaver, *Ecological Relations of Roots*.)

environment, and the large number of roots which run up the slope serve in part as an effective anchorage (Fig. 7).

The half-gravel-slide community represents a distinct successional advance over that of the gravel-slide and forms an intermediate stage between the gravel-slide and the forest. Wide lateral expansion of the root system is prominent in this situation, and at the same time is supplemented by a deep-seated portion which extracts moisture and nutrients from below the 18-inch level, as well as from the second and third foot of soil. The

surface spreading roots may be explained by the frequent occurrence of mountain showers, which give a constant supply of water to the shallow soils. The thick surface layer of loose rock fragments of the gravel-slide are here more disintegrated and closely packed, and this soon gives way to the true soil. Hence evaporation of water is free to take place, and this factor combined with the competition of the taller half-gravel-slide plants accounts for the disappearance of the gravel-slide species.

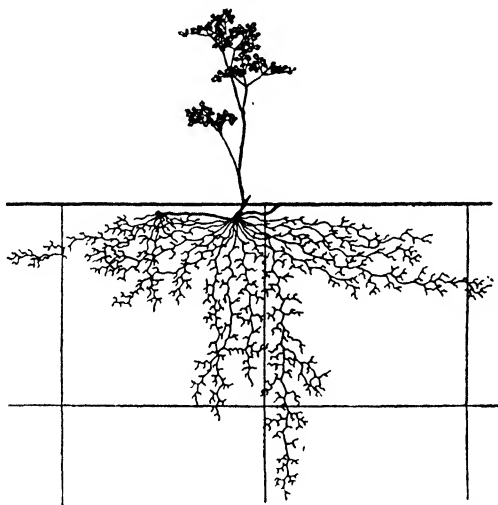


FIG. 8.—*Thalictrum fendleri*, showing the roots extending practically parallel with the surface of the soil with small penetration in depth. (After Weaver, *Ecological Relations of Roots*.)

The final occupants of a half-gravel-slide are a forest community. A number of herbs and undershrubs characteristic of the more mesophytic type of forest, such as *Pirola chlorantha*, *Thalictrum fendleri*, *Erigeron macranthus*, *E. asper*, *Ribes lacustre*, *Rosa acicularis*, *Senecio cernuus* and *Heuchera parvifolia*, were examined. The bulk of the herbs and shrubs of this forest floor are relatively shallow-rooted. Almost without exception the mass of the absorbing system lies within the first 18 inches of the soil surface. Even the roots of the Douglas fir and spruce trees

were found to possess many shallow roots. The forest soil is usually deeper and contains more organic matter than the half-gravel-slide. One or two inches of duff is generally found, and beneath this layer the soil is particularly rich in humus to a variable depth of 8 to 18 inches. Such a substratum furnishes an excellent medium for holding winter rain as well as the frequent summer showers. The shade of the trees reduces the evaporating power of the air, and the loss of water from the soil is further lessened by the layer of duff. The entering water does not penetrate the soil deeply, and the greatest amount of available water is to be found in the first 18 inches of soil. This doubtless accounts for the shallow root habit exhibited by forest shrubs and herbs (Fig. 8).

A number of polydemic species were also investigated, each growing on at least two different habitats. The truth of the theory put forward by Weaver, that water content of the soil determines the nature of the root system, was confirmed in these cases. Thus *Bouteloua*, *Stipa* and *Chrysopsis*, when growing on the plains, were all found to be deep-rooted, and sometimes extended to a depth of 13 feet. When growing on the sandhills they conformed to the root-habit of most of the plants growing in this situation. None of them reached to more than half their former depth, and all showed a marked development of shallow lateral roots. Others, however, such as *Allionia linearis* and *Abronia fragrans*, growing in these same habitats were only very slightly modified. *Euphorbia* and *Yucca* were very frequently modified when grown in half-gravel, although at the same time conforming to the root system; they exhibit when grown on the plains a rather deep and wide-spreading absorbing system. In the former, the extension in depth was always much less, while the branching, like *Yucca*, was much more pronounced (Figs. 9 and 10).

Weaver considered that the water content of the soil offers a logical explanation for this community root habit. In general, root position conforms in a striking manner with the distribution of moisture in the soil, and throughout the whole of this very elaborate investigation only a very few species were discovered,

which showed little or no variation of root system when growing under different external conditions. Since root position so clearly reflects the moisture condition of the soil, especially when interpreted in its community relations, the study of the root systems of plants will greatly increase our knowledge of the value of

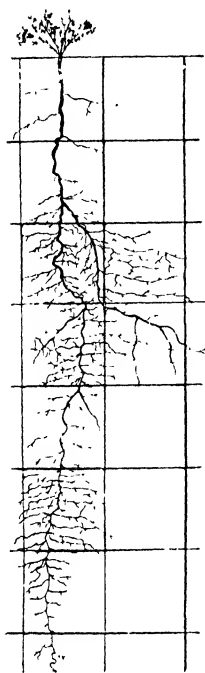


FIG. 9.--The root system of *Euphorbia montana* from the plains. (After Weaver, *Ecological Relations of Roots*.)

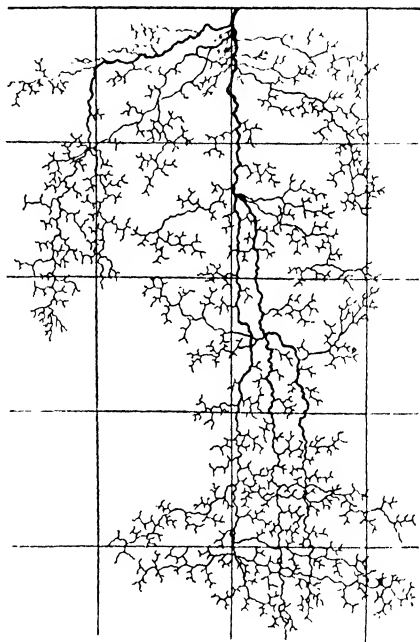


FIG. 10.--The root system of *Euphorbia montana* from the half-gravel-slide. (After Weaver, *Ecological Relations of Roots*.)

different plants in indicating lands of agricultural or non-agricultural value.

Weaver, Jean and Crist (1922) have further extended this work to the root system of crop plants in different types of soil moisture, aeration, etc. The stations chosen were Peru in Eastern Nebraska (rainfall 33 inches); Lincoln, Nebraska (28 inches); Phillipsburg,

Kansas (23 inches) ; and Burlington, Colorado (17 inches). These stations form a decreasing series in rainfall and relative humidity of the air, and so correspond respectively with sublimax prairie, true prairie, mixed prairie and shortgrass and pure shortgrass vegetation. A remarkable correlation was found between the root systems of the crop plants and the natural vegetation. The penetration of the crop plants was extensive. In one case recorded by these observers of a strain of heavy-cropping Kherson oats the roots penetrated to a depth of over 6 feet in Peru, but the maximum development of rootlets occupied the top foot of soil. In Phillipsburg, barley reached a depth of over 6 feet, while *Medicago sativa* (alfalfa) and *Melilotus alba* (sweet clover) extended to approximately 5 feet. At Burlington, owing to the concentration of colloids and carbonates in the soil, the root extension of crop plants was prevented by a hard pan of underlying soil, and less root development was found in consequence. The usual depths for the roots to descend were ascertained to be about 2 or 3 feet, and at the same time there was considerable lateral development.

In the light of their investigations these authors considered that the statements made in agricultural literature to the effect that only the surface soil (about 8 inches) layer is used by roots for absorption, and that the lower subsoil only plays an indirect part, need to be very considerably revised. In fact, in nearly every case, where the roots of crop plants were excavated, the sum total of the development below the cultivated soil surface layer was as big, and in some cases even bigger, than in the surface soil. The dependence of the plant on these more deeply seated layers becomes prominent in times of drought. At such times the vegetation remains unwithered, and fair yields are still obtained from the crops when the surface layer is practically exhausted of moisture.

To determine the depths at which nitrates and water were still absorbed by roots, experiments were carried out by growing crop plants in special containers with wax seals at different levels, and the well-compacted soil at each particular level occupied the same relative level as in the field. Oats were found to absorb water at depths of 2.5 feet, and the amount of water absorbed by barley

was in direct proportion to the downward extension of the root system. *Zea mays* was discovered to absorb water to a depth of 3 or 4 feet of soil, and to absorb smaller amounts after a depth of 5 feet. Considerable quantities of nitrates were removed by roots from the same levels. In every case in which the roots came in contact with a fertilised layer they showed extensive development and branched vigorously, but normal extension into the deeper layers was checked. An important general conclusion arrived at by the authors is that manuring the surface layers of soil where precipitation is small and conditions of drought are ever present is detrimental to crop growth.

The Absorption of Salts

The absorption of salts from the soil is an important problem, and one which is still, unfortunately, in a very unsettled state. The whole question of the permeability of plant cells in general is in a very unsatisfactory position, and largely overburdened with a number of different theories which have been advanced from time to time on the most meagre experimental grounds. Admittedly the problem is beset with difficulties, but the technique which has been developed to investigate this subject leaves a good deal to be desired in most cases. Workers up to the present have been far more interested in portions of the plant and have not troubled to consider the plant as a whole, with the result that the wood cannot be seen for the trees. This result is perhaps unavoidable and to be accounted for by the inherent difficulties of the subject. Only a very limited section of the work on cell permeability can be considered here, and for a very complete discussion of the subject the monograph by Stiles should be consulted.

Plants absorb salts from the soil in dilute solution, and it is only in solution that salts can effect an entrance. The source of the mineral nutrients required by plants is obtained from the soil solution, and this, in spite of its low concentration, appears to be sufficient for the needs of the plant.

A number of investigators have concerned themselves with the

mechanism of entry of salts into plant cells, and a few of the more important of their theories will be now discussed.

Stiles and Kidd (1919) have measured the rate of entry of various salts into slices of carrot and potato. It was found that the carrot answered the purpose better than potato as there was less exosmosis. Each of the slices was 1 mm. in thickness and 1.08 mm. in diameter. The slices were placed in given solutions and the conductivity measured before and after insertion. To counteract the effects of exosmosis, the slices were first placed in distilled water and the conductivity measured after a given time; this value was subsequently subtracted from the final result. The following values were obtained with solutions of sodium, potassium and calcium chloride :—

| NaCl (N/5000) | KCl (N/5000) | CaCl ₂ (N/5000) |
|--------------------|--|--|
| Conductivity fell. | Conductivity fell. In higher concentrations there is a rise in conductivity. | This salt enters more slowly than salts of monovalent metals. Slight losses in conductivity occur. |

Stiles and Kidd endeavoured to determine the concentration on the two sides of the cell wall after complete absorption had occurred. They measured what they termed the "absorption ratio," *i.e.*, the ratio of internal concentration to external concentration. Knowing the size of each disc, they were able to calculate the volume of the cells within the disc, and knowing the amount of salt that had entered they could calculate the concentration within the cells. In weak solutions, it was found that the internal concentration was greater than the external, while in strong the reverse held good. The equation

$$y = Kc^m,$$

in which c represents the external and y the internal concentration, and K and m are constants, was found to hold good for the absorption of salts by the cell. Very much the same type of curve was obtained as for adsorption by a colloid (Fig. 11).

Experiments conducted with aluminium sulphate showed a marked rise in the conductivity of the external medium. One of two explanations may be advanced to account for this result. It

might have been due to the death of the cells under the toxic action of aluminium, with the result that the plasma-membrane was no longer semipermeable and allowed the passage of salts from the interior. The second and correct explanation was that the rise in conductivity was due to the replacement of aluminium ions by hydrogen ions from the cells. It must be remembered in this connection that for every aluminium ion that enters the cell,

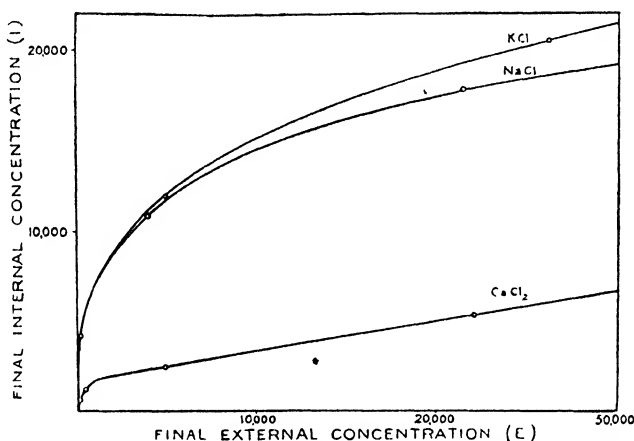


FIG. 11.—The relation between final external concentration in the case of carrot tissue immersed in certain chlorides. (After Stiles and Kidd, *Proc. Roy. Soc. Lond.*)

three hydrogen ions pass out, and since the hydrogen ions are more mobile, a rise in conductivity was found.

Scarth (1925) has observed the entry of ions into the cells of *Spirogyra* by a method depending upon the contraction in length of the chloroplasts, using the long axis and strands of cytoplasm as an index. By this method he discovered that many divalent and trivalent kations are absorbed at first with great rapidity, but the rate of absorption slows down to a standstill. When the initial penetration of ions was plotted against external concentration, the curve rose sharply at first, and then flattened out, and finally fell with higher concentrations. According to Scarth, the penetration of an ion is determined by two opposing reactions

taking place in the interior of the cell—one tending to the absorption of the ion and the other to its exclusion. The latter takes some time to reach its maximum intensity, and in the meantime the former reaches its maximal activity and declines. The sensitivity of both reactions is considered to increase with the valency of the ion. The absorption reaction is said to increase with the atomic weight of the ion in any one chemical group and varies with the solution pressures at the heavy metal end of the series.

The Rate and Order of Entry of Ions into the Cell

The conductivity method has been much used to determine the rate of entry of ions into the living cell. Entrance is shown by a fall in the conductivity. With regard to the order of entry, kations have been found to follow the sequence: K, Ca, Na, Li, Mg, Zn and Al. These are the initial values. At a later time the order of entry is: K, Na, Li, Ca and Mg. The order of entry of anions has also been discovered. Initially, the following values have been ascertained: SO_4 , NO_3 , Cl; while later the order is: NO_3 , Cl, SO_4 . The initial entry of ions seems to depend on their relative mobility, but towards the end of the process it appears to depend on some inherent physiological property of the cell which is quite unknown at present.

In the higher plants it is the root system which absorbs water and dissolved salts from the soil by means of the root hairs attached to the piliferous layer. To a limited extent roots show a selective power of absorption, but at the same time they also take in a number of compounds that do not appear to play any prominent part in metabolism.

As long ago as 1871 Wolff showed for *Lemna* that the salts present in the plant and those in the surrounding medium were not the same, nor were they in the same concentrations. Substances which are present in very minute amount in the external medium are often present in considerable concentration in the plant. Iodine, which occurs in sea-water to the extent of one part in a million, is found in the thalli of the Phæophyceæ in very much

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higher concentration, and at one time these plants formed the sole source of the iodine of commerce. In general terms, the concentration of salts in an Angiospermic parasite are greater than in the host plant :—

| | | |
|--------------------------------|------------------|---------------|
| Poplar | K ₂ O | 6·6 per cent. |
| Mistletoe on Poplar | .. | 16·6 .. |
| Robinia | .. | 2·3 .. |
| Mistletoe on Robinia | .. | 15·9 .. |
| Fir | .. | 8·4 .. |
| Mistletoe on Fir | .. | 30·7 .. |

In the living state the plant is exposed to a very dilute mineral solution—the soil solution—which is also, at the same time, highly ionised, and the problem is to decide whether these various ions enter independently of their partners. This has been found to be the case. Potassium chloride ionises in solution to potassium ion and chlorine ion, but these do not necessarily enter the cell together. In water cultures, for example, a change in reaction takes place with time, the solution becoming either acid or alkaline. This is due to the difference in rate of entry of the various ions composing the solution. Although ions enter the cell independently of their partners, other ions pass out of the cell to replace them. Further, water itself is ionised to a slight extent into hydrogen and hydroxyl ions. This ionisation of water and the alteration in concentration of the ions composing the culture solution accounts for changes in acidity and alkalinity.

Redfern (1922) ascertained in the case of pea seedlings grown in culture solution of calcium chloride that the rate of absorption of calcium and chlorine ions was very different :—

| | Calcium. | Chlorine. |
|--------------------------|-----------|-----------|
| | Per cent. | Per cent. |
| After 36 hours | 17·7 | 3·6 |
| „ 40 „ | 12·8 | 4·0 |
| „ 60 „ | 11·6 | 3·9 |

It will be seen that the calcium ions entered the cell very much more rapidly than did the chlorine ions. Theoretically the solution should have shown an acid reaction, owing to the combination of the excess of chlorine ions remaining in the external solution with the hydrogen ions of the water. This, however, was not found to be the case, as potassium and magnesium from the root cells replaced entering calcium.

Stiles (1924) has shown that when storage tissue was immersed in a solution of a single mineral salt, the ions of the salt are not necessarily absorbed in equivalent amounts, the balance of the ionic changes in the external solution being maintained by the diffusion of ions out from the tissue. Absorption of either ion proceeds fairly rapidly at first, but after some four to five hours the rate of absorption falls off greatly. The absorption of each ion proceeds toward an equilibrium condition, which is not that of the equality of concentration of the ion within and without the tissue, but which is dependent on the concentration. The absorption ratio varies continuously from a fraction of unity to many times unity with decreasing concentration of the salt, and the position of equilibrium is given with fair approximation by the absorption ratio discussed above. The process of absorption is therefore a complex one, and indicates that the absorption of salts by living cells is not a simple process of diffusion. Two explanations are possible: either the salt combines chemically with some cell constituent or the salt or its ions are adsorbed at the surface of some cell constituent or constituents. The latter appears the more reasonable suggestion. The protoplasm of the cell is a colloidal system, and there is evidence that the material in the vacuole is also colloidal in nature so that the plant cell may well act as an adsorbent of inorganic salts. Similarly, Haas and Reed (1926) found for *Citrus* seedlings that the two ions were not absorbed in equivalent quantities. In nearly every case more kation was absorbed than anion—a result confirming the work described above. The amount of ion absorbed depended on the nature of the other ion of the salt. Thus more kation was found to be absorbed from the nitrate than from the chloride of the same metal. The absorption of calcium was discovered to be retarded

by the presence of potassium, and sodium produced a similar but less intense effect. Potassium and nitrate were generally absorbed in large quantities, but the presence of chloride hindered the absorption of potassium. In all cases an exchange of ions between roots and solution was observed, and this caused a marked change in the pH of the external solution. Jacobson (1925) found that wheat plants 100 days old could change the reaction of the culture solution from an original pH of 3.9 to 6.3. He attributed this change in acidity to the selective absorption of nitrate anion from potassium nitrate, the potassium kation being left, and, in part, also to root excretion. With rice, on the other hand, the reverse change was found to exist. Rice plants reversed a culture solution of pH 5.0 to 3.0 in three days. This is attributed to the greater absorption of kation from sulphate, the anion being left behind, and to the excretion of carbonic acid by the roots.

Antagonism

The question of mineral salt entry into the plant is complicated by the phenomenon of antagonism. It has been known for a considerable time that solutions of single salts, especially magnesium salts, are highly toxic to plants. Loeb, for example, in the early years of this century was able to show that the eggs of *Fundulus* were inhibited in their development if they were placed immediately after fertilisation into a pure solution of sodium chloride having the same concentration as sea water. If, however, small amounts of the salt of a divalent metal such as calcium or magnesium were added, the development of the embryo proceeded normally. If the outer egg membrane were damaged or torn, antagonism was no longer exhibited. Loeb therefore concluded that the seat of these antagonistic activities must be located in the cell membrane. A number of salts, when present in solution, appear to be able to antagonise the toxic effects of each other. This phenomenon is spoken of as "antagonism of salts," and such solutions are termed "balanced solutions." Sea water is a typically balanced solution. Osterhout showed a number of years ago that water culture solutions are really balanced solu-

tions. The various salts present are in themselves toxic to the growth and well-being of the plant, but when mixed together are quite harmless, and appear to antagonise the toxic effects of each other. Not all single salt solutions are toxic to the same degree. Of the chlorides of potassium, sodium and calcium, sodium is the most toxic and calcium the least toxic to wheat seedlings. Trelease and Trelease (1925, 1926) tried a wide range of concentrations of single salt solutions on the growth of roots in wheat seedlings, and found in all cases that magnesium was the most toxic in its action. The concentration of the single salt solutions also markedly affects their toxic action. Thus potassium nitrate in low concentrations was less toxic than calcium nitrate, but in higher concentrations the reverse held good. Temperature also influenced the action of the salt. The greatest variation in the growth of wheat seedlings was found at 30° C. in single salt solutions. Eisenmenger (1928) has shown that the order of toxicity is different for different degrees of toxicity. Thus, for slight growth retardation of the roots of wheat seedlings, the order of the three salts is KH_2PO_4 , $\text{Ca}(\text{NO}_3)_2$, MgSO_4 , whereas for high retardation of the growth rate, the order is MgSO_4 , KH_2PO_4 , $\text{Ca}(\text{NO}_3)_2$.

Antagonism has also been found to exist between aniline dyes and electrolytes. Irwin (1926, 1927) has suggested a method whereby it is possible to separate the action of the electrolyte on the protoplasm from the action of the dye. The tissue is placed for a given time in the salt solution, and this is followed by immersion in a solution of the dye which contains no salt. The penetration of dye into such cells is then compared with the controls (cells which have not previously been immersed in salt solution). From these observations it is possible to arrive at the effect of the salt on the protoplasm, since no error is introduced by the salt adhering to the surface of the cell wall, nor does the salt diffuse out of the cell. In the second place, cells previously exposed to distilled water or salt solution are immersed in dye solution containing the dissolved salt and the rate of penetration of dye compared with the controls (cells which have previously been exposed to distilled water and then placed in the dye solution

containing no salt). With cresyl blue the penetration of this dye was delayed when it was exposed to the action of monovalent kations, *e.g.*, sodium chloride, before they were placed in the dye solution. When a monovalent salt was mixed with the salt of a divalent metal, *e.g.*, magnesium chloride, antagonism was shown and the entrance of the dye was not hindered. The previous immersion of the cells in solutions of di- or tri-valent metals produced no effect on the entrance of the dye.

It has already been stated that ordinary water cultures are really balanced solutions. In the case of experiments on the effect of different elements on growth, and in which one element is deliberately excluded from the water culture, a double result is actually obtained. In the first place the plant is deprived of a particular element, and in the second the physiological balance of the solution is altered at the same time.

Zwaardemaker and his co-workers (1916 to 1920) have made some highly important contributions to this phenomenon of antagonism. Actually the work was carried out with frogs, but, nevertheless, it is of considerable significance to plant physiologists in its general aspect. It has long been known among animal physiologists that when a frog's heart is perfused with Ringer's solution¹ it will continue to beat for a very considerable time. If, however, a solution of sodium chloride is used alone, the beats stop at once. On the addition of the other salts composing the complete solution, the beats continue once more. Ringer's mixture is a typically balanced solution. It was originally shown by Ringer that the salts of the elements caesium and rubidium, which occur in the same group of the periodic table as potassium, were able to replace the latter in his mixture. Of all the elements present in this solution, potassium is the only one which exhibits feeble radioactivity and expels β -rays. It is many thousand times less radioactive than uranium, while radium is 10^9 times as active. Rubidium is even less radioactive than potassium, and radioactivity has been denied for caesium by many. It was shown by Zwaardemaker and Feenstra (1916) that a frog's heart will beat

¹ Ringer's solution is made up in the following proportions : NaCl, 0.67 per cent. ; KCl, 0.01 per cent. ; CaCl₂, 0.02 per cent. ; NaHCO₃, 0.02 per cent.

equally well in a Ringer mixture which contains, instead of potassium chloride, a calculated quantity of uranium nitrate, thorium nitrate, radium bromide or niton, equivalent in radioactivity to the amount of potassium in normal Ringer's solution. In other words, a frog's heart which has stopped beating in a potassium-free solution will recommence to beat when an equivalent quantity of a radioactive element is added to the fluid. They also found that a heart could be made to beat when it was exposed to β -radiation from mesothorium or radium at a distance of 1.2 cm. These original experiments were carried out in winter, and it was found that in summer smaller quantities are sufficient and the reduction in amount may be brought about by the addition of eosin or fluorescein to the solution. In any case, the reduction in amount appears to be due to the better adsorption of the radioactive element by the endothelium of the frog in summer. The following paradox is explained by the assumption that electrically charged particles are adsorbed. It was found, for example, that the various methods of keeping the heart pulsating or restoring its beat when it had stopped may be arranged in two classes :—

| <i>Group I.</i> | <i>Group II.</i> |
|---------------------|------------------|
| Potassium. | Uranium. |
| Rhubidium. | Thorium. |
| Cæsium. | Radium. |
| β -Radiation. | Niton. |

A heart beating under the action of one of the elements in the above two classes will continue to beat when it is supplied with another member of the *same* group, but comes to a standstill if supplied with an element from the other group. Thus, either β -radiation-Ringer or radium-Ringer will restart a heart stopped by the use of a Ringer solution free from a radioactive element. In the case of β -radiation-Ringer solution the heart will continue to beat if the β -radiation be replaced by either cæsium or rhubidium, but stops at once if it be supplied with uranium or thorium or any other member of the second group. Before this "switching-over" from one group to the other can take place, the heart must

be well washed with a solution free from radioactive elements. According to physicists, in Group I. we are dealing with elements which are expelling negatively charged particles, whereas the elements in Group II. are discharging positive α -particles. In radium both kinds of particles are discharged, but the α -particles predominate. The adsorption of either kind of particle gives to the heart the necessary electric charge, but in a mixture of the two groups the two different kinds of charge will neutralise each other and the necessary electrical condition cannot be maintained.

An interesting botanical analogy has been discovered by Zwaardemaker in this connection. The centres of plates containing luminous bacteria were exposed to mesothorium, which expels α -particles and β -radiation from polonium, and the cultures were subsequently photographed by their own light. In both cases it was found that the centres of the plates were black where local death of the bacteria had occurred in which the two types of ray had neutralised one another.

Many claims have been made that radioactivity markedly increases the growth of crops, and preparations alleged to contain radioactive elements have been placed on the market from time to time as being suitable for plant growth. Unfortunately the evidence on this matter is very conflicting and requires further investigation. Should radioactivity prove to stimulate plant growth, it may be due to some such action as the radioactive elements have on the frog's heart.

The Mechanism of Antagonism.—The mechanism of antagonism is still obscure. It is known that mixtures of salts enter a cell more slowly than single salts. Loeb, therefore, considered that the whole matter was due to the keeping out of salts from the cell. The mere slowing down of the rate of entry will not explain the matter. However much the penetration is reduced, the final result will be unaffected. The most probable explanation is that the proteins of the protoplasm adsorb salts in certain definite proportions, and unless the salts are adsorbed in these definite ratios, the proteins are no longer in the active state. Osterhout has brought forward a so-called "dynamic" explanation of antagonism based on his experiments with *Laminaria*. He assumed

that two processes are involved, one producing a fall in the resistance of the tissues and the other a rise, and represented the two processes in the following scheme :—



in which A breaks down to give M, and M by further decomposition gives B. He at the same time assumed that the electrical resistance of the protoplasm is due to M, and that the resistance of the tissue is proportional to M plus some constant equal to the resistance of the tissue when dead. Under natural conditions, such as the life of *Laminaria* in the sea, it is supposed that the velocity constant of these reactions remains constant, but when transferred to a single salt solution or to a mixture of two salts, the velocity constant of the reaction $A \rightarrow M$ and $M \rightarrow B$ becomes in some way markedly altered. If, for example, the reaction $A \rightarrow M$ is more rapid than $M \rightarrow B$, the material M will accumulate and the resistance will gradually rise till A is exhausted, when M will be gradually more and more slowly formed. Finally, M will decompose faster than it is formed, and a rapid decline in the resistance will follow. This theory assumes a great deal more than is warranted by the facts, and takes no account of the complex nature of the system involved.

The Effect of Temperature on Absorption

With tissues of potato and carrot, Stiles and Jørgensen (1915) ascertained that the relation between absorption of hydrochloric acid and temperature was practically logarithmic and the expression representing the absorption rate was approximately

$$\frac{dx}{dt} = K(A - x),$$

where $\frac{dx}{dt}$ was the rate of absorption at any time when x represented

the diminishing concentration of acid in the external medium and A the original concentration of acid. Roughly speaking, it was found that for every rise of temperature of 10 degrees the rate of absorption of hydrogen ions was increased 2.2 times. Whether

this result is to be correlated with any definite chemical reaction or is merely due to adsorption is difficult to say.

✓Sen (1928) found that the effect of increasing temperature was not, as might be expected, to produce a steady increase in permeability. The effect, for example, of an increase in temperature between 20° C. and 30° C. to 35° C. was to bring about an increase in permeability, but the rate of increase fell away, and frequently no change in permeability occurred with a further rise in temperature. Beyond 35° C. and 40° C. there was a decrease in permeability till death supervened and the plasma-membrane had completely broken down.

The Mechanism of Entry of Salts into the Cell

The study of the mechanism of entry of salts into the living cell has led to the production of endless theories of varying worth. Osterhout (1925) and Osterhout and Dorcas (1925-26) have advanced the view that the penetration of living protoplasm is confined to undissociated molecules and that ions are unable to enter. Brooks (1926) was unable to find any support for this theory, while Hoagland, Hibbard and Davis (1926) found in the case of *Nitella* that the cells are able to take up bromine from dilute solutions of potassium bromide without damage to themselves, and that the final concentration of the bromine within the cells might in some cases be as high as sixty times that of the external medium. This process could only take place in the light, and the temperature coefficient was found to be comparable with that of a chemical reaction rather than with that of a simple diffusion process. In view of the very dilute solutions that were used in this work, the authors preferred to interpret their work as involving the absorption of ions rather than undissociated molecules.

It is very unfortunate that the majority of work on cell permeability has been conducted with solutions of relatively high concentration. The soil solution with which the plant is primarily concerned is very dilute, yet the concentration within the cells is high. It is difficult to account for such a difference of concentration on the two sides of the cell wall.

Hoagland and Davis (1925) placed equal masses of healthy cells in slightly acid solutions of potassium chloride (0.001 M). Some of the cells were placed in the light and some in darkness, and others again in daylight for varying periods of time, such as one, three, five and eight hours, as well as the whole period of daylight. After the lapse of a few days the amount of chlorine was determined, and a very definite correlation was discovered between absorption and illumination. Only very slight absorption occurred in the absence of light or if the period of illumination were short. These authors, therefore, considered that the energy derived from light is directly concerned with the penetrations of substances into the cell. In roots, the majority of which live in complete darkness, the energy for this purpose is considered to be due to the breakdown of carbohydrate derived from photosynthesis. It was discovered in the course of these investigations that only normal cells will behave in this way. It is well known that if cells are injured, the salts diffuse out, *i.e.*, the cells possess a one-way permeability system and the matter is not a simple case of the Donnan equilibrium. "It seems essential to recognise that plants, and, no doubt, living cells in general, have the power to bring about movements of solutes against a concentration gradient and that the phenomenon is not limited to certain definite organs, such as the kidney in the animal body" (Hoagland and Davis). This is simply another and more elaborate method of saying that the living plant cell has a permeability mechanism which we do not understand.

It was shown by Redfern (see above) that the two ions of a salt do not enter a cell in equivalent proportions, and this work has been confirmed by a number of other investigators. Hoagland and Davis found that one ion may retard or accelerate the absorption of another. Thus, the sulphate ion only enters the cell slowly and is able to hinder the entrance of potassium, so that with solutions of equal concentration, barley will absorb potassium very much more rapidly from a potassium chloride solution than it will from a potassium sulphate solution. The hydrogen-ion concentration of the medium may also play a part in cell permeability. Thus the nitrate ion penetrates the cells of *Nitella* very

much more rapidly in acid solution than in an alkaline one, and a similar state of affairs has been found for the chlorine ion. Barley and cucumber remove nitrate anions in greater proportion from solutions with an acid reaction (pH 5.0) than from those showing an alkaline reaction. On the other hand, a greater proportion of cations are taken up from solutions with a high pH . Such factors as these all help to complicate an already difficult problem.

In bringing such a discussion as this to a close, it cannot be said at the present time that our knowledge of cell permeability extends to much more than a mass of empirical facts, while our knowledge of the nature of the plasma-membrane and the mechanism of absorption is very meagre. The whole subject of permeability is unfortunately suffering from being overburdened with a large number of theories, and it is to be regretted that each new investigator feels it necessary to add fresh burdens, which are in many cases scarcely in accordance with the experimental facts, or may even be cases of special pleading.

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CHAPTER II

TRANSPIRATION—THE TRANSPIRATION CURRENT— TRANSLOCATION—ROOT PRESSURE

Definition of Transpiration—Cuticular and Stomatal Transpiration—Factors Affecting Transpiration Rate—Relative Transpiration—Stomatal Regulation of Transpiration—Mechanism of Stomatal Movement—Effect of Light on Transpiration from the Mesophyll—Importance of Transpiration to the Plant—The Transpiration Current—Cohesion Theory of Ascent of Sap—Bose's Theory of Ascent of Sap—Translocation—Passage of Stimuli in Plants—Root-Pressure.

THE transpiration of a plant may be defined as the evaporation of water from its aerial parts, such as the stem, twigs and leaves. The amount of water lost by this method is in many cases considerable. Balls has estimated that the loss of water from an Egyptian cotton crop by transpiration is about 50 tons per acre per day or 3 pints per plant.

The main mass of transpiration takes place from the leaves, but it has also been found to occur in the stem as well as in the twigs.

It is well known that transpiration may be divided into two classes: *cuticular* and *stomatal*. Cuticular transpiration is the amount of water lost through the cuticle of a plant; stomatal transpiration is the loss of water through the stomata. In ordinary mesophytic plants stomatal transpiration represents 80 to 97 per cent. of the total water-loss, whereas cuticular transpiration only represents about 2 to 3 per cent. of the total loss. It has been found that even the very thickest cuticles transpire to a certain extent. In some leaves the cuticular and stomatal transpiration is approximately equal. A good example of this is to be found in the Jamaican rain forests, where the cuticular

transpiration represents 58 per cent. of the total transpiration of the leaves.

Shreve (1920), working with *Encelia farinosa*, has found that the leaves possess the power of cutting down their relative transpiration ¹ during the months in which aridity is increasing. The plant possesses two distinct types of leaves. A mesophytic type of leaf is present during the cool months of the year. This is shed when arid conditions arrive, and is in turn succeeded by a xerophytic form. Curiously enough, it was ascertained that when discs of uniform section were cut from the two types of leaves, those obtained from the xerophytic leaves lost water 1.44 times more rapidly than discs cut from the mesophytic form; although if the water-loss were calculated on a dry weight basis the discs from the xerophytic leaves only lost water 0.78 times as fast as the mesophytic discs. On the other hand, Shreve discovered that while arid conditions were in existence the xerophytic leaves secrete a viscous brown fluid which appears to play a considerable part in hindering excess of transpiration.

In stomatal transpiration the diffusion of water vapour takes place through the open pores of the stomata. The physical side of the matter has been very fully investigated through the now classical investigations of Brown and Escombe, Renner, Jeffries and others, and will not, therefore, be considered here. Since, however, the main mass of the transpiration of a plant takes place through the stomata, it is obvious that external factors play a very considerable part in determining the rate of water-loss. Stomatal diffusion is a process of evaporation. Evaporation from the wet cell walls of the mesophyll cells takes place, and is markedly affected by the external conditions of the atmosphere prevailing at the time. Energy is expended in the process, and it was early shown by Brown and Escombe that 50 per cent. of the incident sunlight falling on the leaf of *Polygonum Weyrichii* was used in transpiration, and only some 1 per cent. or less utilised for photosynthesis.

Factors Affecting the Transpiration Rate.—The factors affecting

¹ Relative transpiration is the amount of transpiration (T) divided by the evaporating power of the air (E), i.e., T/E, see p. 87.

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transpiration may be divided into two classes: external and internal.

External Factors.

- (a) Humidity of the atmosphere.
- (b) Wind.
- (c) Temperature.
- (d) Barometric Pressure.
- (e) Light.

Internal Factors.

- (f) Stomata.
- (g) The water-content of the mesophyll tissue.

The Humidity of the Atmosphere.—If the atmosphere surrounding the leaf be saturated with water-vapour, no gradient of water concentration can be set up between the intercellular spaces of the leaf and the external atmosphere. In such circumstances no evaporation can take place, and, in consequence, transpiration will be greatly diminished.

The main concern in such a question as this, is the water deficit of the atmosphere and the temperature must be known for a knowledge of this factor. Any rise in temperature will alter the water vapour pressure and cause an increase in the water deficit. Similarly, a decrease in temperature will cause a decrease in the water deficit.

If transpiration be nothing more than a process of evaporation from the wet walls of the mesophyll tissue, there should exist a simple relation between the moisture present in the atmosphere and the transpiration rate.

Francis Darwin (1914) made the first attempt to ascertain for *Hedera helix* the relationship between the transpiration rate and the humidity of the atmosphere when the temperature was kept constant. He discovered that with decrease in humidity there was increase in the transpiration rate, and that a straight line graph was obtained, *i.e.*, there was a simple linear relation between the two. The line, however, when produced backwards, did not

cut the axis at the 100 per cent. saturation point, but at the 105 per cent. saturation point. The explanation of this curious result proved simple. The living plant respire in the course of its metabolic activities, and the temperature in the immediate vicinity of the mesophyll cells will on this account be higher than that of the surrounding atmosphere. Hence the air in the direct neighbourhood of the mesophyll cells will not be saturated when the external atmosphere is in that condition. It is on this account that the value 105 per cent. was obtained instead of the expected 100 per cent.

From the results of these experiments, Darwin was able to

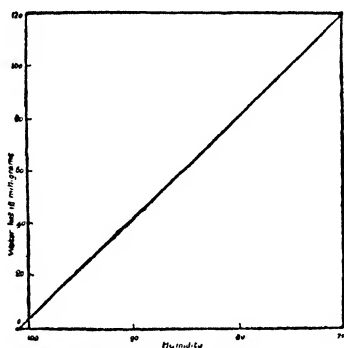


FIG. 12.—Effect of changes of humidity on the transpiration of *Hedera helix*. (After Henderson, *Ann. Bot.*)

calculate the temperature of the mesophyll compared with the surrounding air. He worked at 16° C. and the vapour pressure at this temperature is 13.51. This value, plus 5 per cent., gives a vapour pressure of 14.2. The corresponding temperature to this vapour pressure is 16.8° C. Thus the mesophyll cells were 0.8° C. higher in temperature than the surrounding air.

These observations of Darwin have been in large measure confirmed by Henderson (1926) using more controlled conditions. He found that in *Hedera helix* his curve (Fig. 12) did not cut the axis as far back as Darwin's, and that the temperature of the mesophyll cells was 0.4° C. and not 0.8° C. higher than the surrounding atmosphere. He was further able to show that the

effect of changes of humidity on the rate of transpiration for higher humidity values was to make the surface of the cell act as a damp surface in a purely physical way, since the graphs follow within fairly narrow limits the equation for change in rate of water-loss with change of humidity :—

$$E_1 = E \left(\frac{S_{T_1} - \frac{y}{100} \cdot S_{t_1}}{S_T - \frac{x}{100} \cdot S_t} \right)$$

where E is the evaporation rate, S_T is the saturation vapour pressure of air at leaf temperature T° , $\frac{x}{100} \cdot S_t$ is the percentage of saturation water vapour pressure at a temperature t° , E_1 is the rate of evaporation when S_{T_1} is the saturation vapour pressure of the air at leaf temperature T_1° , and $\frac{y}{100} \cdot S_{t_1}$ is the percentage of saturation of the water vapour pressure at air temperature t_1° .

Wind.—The effect of wind on the transpiration rate is to remove the layers of saturated air or partially saturated air which cover the leaf surface. This will lead to an increase in the transpiration rate.

Temperature.—In connection with transpiration, temperature has also to be taken into account. Temperature plays no direct part in affecting the water-loss from leaves, but acts in the indirect capacity of affecting the saturation deficit of the atmosphere. Increase of temperature will lead to an increase of the saturation deficit and thus to an increase in the rate of transpiration.

Humidity, temperature and wind can all be expressed in terms of the evaporating power of the air, and it is a comparatively simple matter to determine at what rate water is evaporated from a surface.

Light.—Light materially affects the transpiration rate. When, however, the effect of light is being investigated, it is of the utmost importance that all the other factors involved are kept strictly constant. The matter becomes difficult when investigations are

conducted under field conditions in the open. It is therefore more convenient for comparison to use a record of the rate of evaporation from a standard water surface at the same time. This expression summarises the influence of the prevailing atmospheric conditions and makes it an easy matter to detect any differences between the effects of these on transpiration rate and evaporation rate.

Some years ago, Livingston (1906) suggested a method of measuring the evaporating power of the atmosphere, a value which he termed E . If T represented the transpiration rate, the ratio T/E , referred in both cases to unit area, was termed by him the "relative transpiration." Relative transpiration eliminates the direct evaporating power of the air and the physiological behaviour of the leaf is exhibited.

The main difficulty in this connection is to measure E in a satisfactory manner. It would, of course, be possible to place an uncovered basin in the open and find the loss of water by weighing at intervals. This method, however, has several disadvantages in actual practice. One of the main sources of error in such a procedure is this: the rate of evaporation will alter as the water level falls below the rim of the vessel. Livingston escaped from the difficulty by means of a device known as the "porous cup-atmometer." Various types of these are now in use. A common variety consists of a flask fitted with a cork and glass tube at the other end of which is attached a porous porcelain candle. Flask, tube and porous candle are filled with water, and the evaporation determined by weighing at intervals. Another method of comparing T/E is by means of Pische's device. This, in essence, is very simple. It consists of a piece of filter-paper placed over the mouth of a tube containing water. It is not, however, very convenient to use in the field.

The question arises as to how far the atmometer responds in the same way as the leaf. Livingston assumed that by his conception of relative transpiration the direct effect of the atmospheric conditions on the transpiration rate could be neglected in the interpretation of his results. The main assumption underlying this principle is, that changes in the atmospheric environment

affect equally the rate of transpiration of a plant and the rate of evaporation from a water surface. As Knight (1917) has pointed out, the assumption is scarcely warranted by the facts. Livingston (1915) and Briggs and Shantz (1917) have shown that atmometers of different sizes and shapes are not strictly comparable under changing conditions. For example, a change in environment which doubles the rate of evaporation from one atmometer does not necessarily double the rate from another of different size or shape. In exactly the same way the size or shape of the leaf may modify the influence of changes of external conditions on its transpiration rate.

It appears, therefore, that it is not possible to compare one atmometer with another, or one leaf with another under changing external conditions, so that it seems scarcely justifiable to compare an atmometer with a plant. A further criticism advanced by Knight is to be found in the structure of the leaf. A portion of the path along which the diffusion stream passes from the evaporating mesophyll cells to the outside air, is situated actually within the leaf and, being protected by the epidermis, is not, therefore, subject to the direct influence of movements of the outside air. In the case of the atmometer, however, the whole of the diffusion stream is exposed to air movements, and it is theoretically possible by means of a sufficiently rapid current of air to reduce the moisture in the air close to the evaporating surface to the same concentration as that in the general atmosphere. In the case of the leaf, it is only at the surface of the epidermis and not of the evaporating mesophyll that this minimal concentration can be obtained, so that it is to be expected that changes in the speed of air movement will have less influence on the transpiration rate of a leaf than on the rate of water-loss from an atmometer.

From a large series of experiments, Knight has shown that changes in the velocity of wind affect the plant and atmometer in different ways, and it is only when the air movements are maintained at a constant value that this method of comparison by use of relative transpiration can be used to eliminate changes in relative humidity and temperature on transpiration; since it has been found that these two factors act equally upon transpiration

from a plant and evaporation from an atmometer. It is clear then, that *only when wind velocity is constant* does relative transpiration give a satisfactory measure of the intrinsic transpiring power of a plant.

A number of years ago Livingston (1906) compared the transpiration of the succulent *Euphorbia capitellata* with the evaporating power of the air, and further ascertained the relative transpiration (T/E) at the same time. An examination of his curves (Fig. 13) show a general similarity, but with an important difference. The highest point of transpiration was found to occur between 10 a.m.

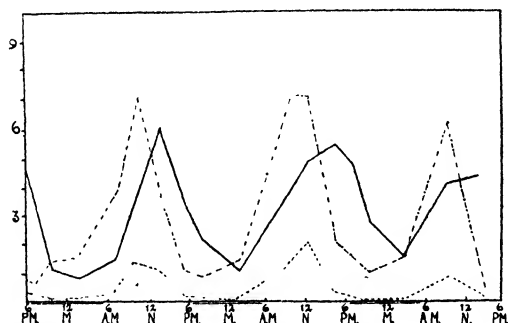


FIG. 13.—Transpiration of *Euphorbia capitellata* through three days compared with evaporation (continuous line) and relative transpiration (dot-dash line). The scale of the ordinates is different in the three graphs. (After Livingston, modified. From Skene, *Biology of Flowering Plants*.)

and 12 noon and this increase was followed by a steady decrease. The evaporation rate, on the other hand, increased between 2 p.m. and 4 p.m., at which point the maximum was reached. The prevailing external conditions are evidently of such a nature as to cause maximum evaporation in the late afternoon, whereas some reaction in the plant has led to a diminution of water-loss at an earlier time. The same result is reflected in the graph of relative transpiration. If both the transpiration and the evaporation had been affected in a similar way, this latter curve should have been a straight line. The actual result obtained shows that transpiration is increasing more rapidly than evaporation till the maximum is

reached, and the subsequent increase in the evaporating power of the air is not reflected in the transpiration. A further point in this connection is that transpiration increases greatly in light, and falls in the dark (Fig. 13). The light produces some considerable change in the leaf, and, in actual fact, brings about an increase or

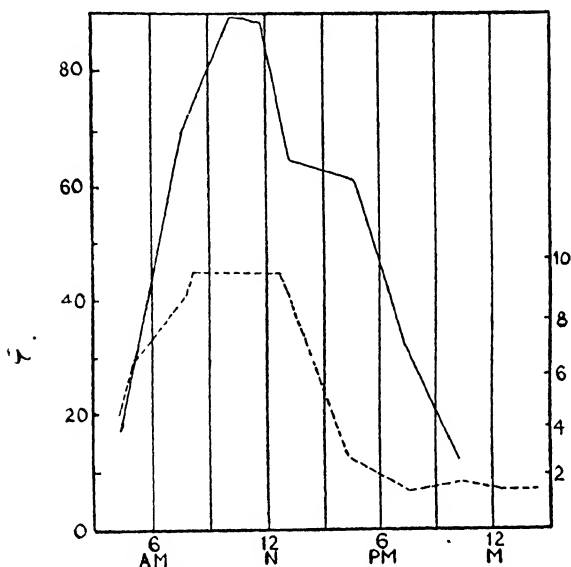


FIG. 14.—Transpiration and stomatal movement. The continuous line represents the transpiration of *Verbena ciliata*, the broken line represents the extent of opening of the stomata in microns. (After Lloyd, modified. From Skene, *Biology of Flowering Plants*.)

decrease in the size of the stomatal openings through which the main mass of transpiration is taking place.

Stomatal Regulation.—The question as to whether stomatal aperture does or does not control the transpiration rate has led to a good deal of controversy which has only recently been settled. Lloyd (1908), at the end of his elaborate experiments on the relation between stomatal opening and transpiration rate, came to the conclusion that there was no correlation between them (Fig. 14). Trelease and Livingston (1916) arrived at a similar

coincident increase of osmotic pressure. It was shown by Leitgeb, however, that the stomata open in the light in the absence of carbon dioxide, while the later experiments of Lloyd (1908), Iljin (1915, 1922) and Loftfield (1921), showed that the opening in light is accompanied by a decrease in the starch-content of the guard-cells, and at closure the starch-content is increased. In other words, the behaviour of the guard-cells was the direct opposite to that of the assimilating cells of the mesophyll. Such being the case, the decrease in the starch-content must lead to a considerable increase in the osmotic pressure of the guard-cells giving the necessary conditions for stomatal opening. Wiggins (1921), in an important publication, ascertained the osmotic pressure of the guard-cells and the epidermal cells at different times during the day. With *Cyclamen* the osmotic pressure rose from 14.6 atmospheres at 7 a.m., to a maximum of 31.0 atmospheres at 11 a.m., and then gradually fell to 18.5 atmospheres at 5 p.m. The epidermal cells, on the other hand, remained at a constant value of 10.2 atmospheres throughout. Similarly, in the beet, the epidermal cells showed a constant osmotic pressure of 12.5 atmospheres, while the osmotic pressure of the guard-cells rose from 23.5 atmospheres at 7 a.m. to 31.6 atmospheres at 11 a.m. It remained constant at this value till 1 p.m., and then gradually fell to 25.0 atmospheres at 5 p.m. Similar results were obtained by Sayre (1923) for *Rumex patientia*. The guard-cells at night when the pore was closed showed an osmotic pressure of 13 to 14 atmospheres, when fully open at noon, it reached a value of 23 atmospheres. The subsidiary cells showed a constant value of about 15 atmospheres, and the other epidermal cells about 13 atmospheres.

Lloyd, Loftfield and others, have supposed that the change of starch into sugar and sugar into starch is in the nature of a reversible enzymic reaction. The question arises as to how far does the influence of light affect the direction of the equilibrium. Leitgeb, F. Darwin, Lloyd, have shown that a reduction in the partial pressure of the carbon dioxide surrounding the leaf led to the opening of the stomata, while Lindbauer (1917, 1926) discovered that increase in the partial pressure of the gas led to the

opposite result. Weber (1923, 1926, 1927A, 1927B) connected these apparently contradictory facts with the action of the light, and assumed that the concentration of carbon dioxide in the guard-cells was regulated by the rate of its photosynthetic absorption. ✓ Both Lindbauer and Weber have shown that remarkable physiological reactions accompany the functioning stomata. The plastids changed their position, the nucleus underwent changes in size and shape and the open and closed stomata showed differences in their power of reducing silver nitrate. ✓

More recently Sayre (1923) and Scarth (1926) have shown that these various changes observed in the guard-cells are secondary phenomena of a simple common case. Using solutions which readily penetrated the guard-cells, such as solutions of ammonia and acetic acid, Scarth found that the stomata opened in both alkali and acid, but more fully in the former. In an intermediate range of pH (5.5 to 7.0) for *Zebrina pendula*, the stomata remained closed, but opened in increasing concentrations of both alkali and acid up to the limits of injury. It was further discovered that starch made its appearance in the pH zone of closure and disappeared in that of opening, and these changes were reversible. Scarth also ascertained that the sap of the guard-cells under normal conditions possessed a higher pH (i.e., were on the alkaline side) when the stomata were open, and that a low pH was registered when they were closed. The range appeared to stretch from pH 7 to 4.5.

Sayre obtained very similar results for *Rumex patientia*. The guard-cells showed a more alkaline reaction when the stomata were open, and the movement could be controlled at will by variations in the pH of the guard-cells. The stomata opened within the range of pH 4.2 to 4.4 and closed on both sides of this. Sayre considered that there was an optimum pH for the enzymic hydrolysis of starch, whereas Scarth thought from the fact that there are two widely separated optima, and that the direction and rate of the reaction appear to have no relation to the relative concentrations of the reagents, that stomatal opening is not explicable on a simple enzymic basis.

The metabolism of the stomata of white-margined *Pelargonium*

leaves has been investigated by Kümmler (1922), who showed that they contained abundant starch—more, in fact, than the guard-cells of normal green leaves—and that they opened in light and closed in the dark. Very wide opening only occurred if the water supply were highly favourable. These results appear to show that chlorophyll is not an essential part of the plastids of the

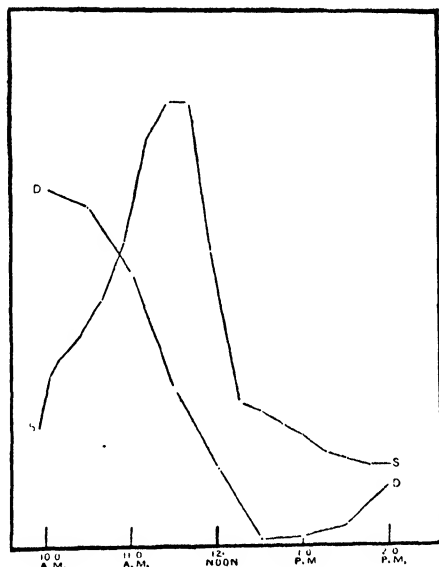


FIG. 21.—Graphs showing the continued opening of stomata in presence of increasing water-loss. S = stomatal aperture; D = water deficit. (After Knight, *Ann. Bot.*)

guard-cells, and it is possible that they are in the nature of leucoplasts.

External conditions, such as humidity, temperature, etc., all play a part in influencing stomatal opening. Loftfield (1921) has shown that temperature markedly affects stomatal movement and that for a rise of 10°C. the rate of movement is approximately doubled. Excessive transpiration may lead to a loss of turgor by all the cells of the leaf, including the guard-cells, when collapse and closure of the stomata takes place. ✓

✓The investigations of Knight (1917) have largely cleared away the conflicting statements that have been made from time to time concerning the effect of wilting on stomatal behaviour. F. Darwin (1898) considered that the stomata opened widely in the early stages of wilting, and Knight has confirmed this observation. When the leaf is losing water more rapidly than water is supplied to it, there is a temporary increase in stomatal opening (Fig. 21). Only when the wilting is very pronounced does closure take place. ✓

The complexity of the stomatal mechanism is shown by the recent work of Maskell (1928), who discovered a well-marked rhythm in the stomata of *Prunus laurocerasus*. Under constant artificial light intensity the stomata began to close towards the evening and to open about midnight (for a full description of these experiments, see Chapter III.).

It must be confessed that our knowledge of the underlying mechanism which controls and regulates stomatal behaviour still leaves much to be discovered.

The Effect of Light on Transpiration from the Mesophyll.—Although a great deal has been done in the way of discovering the effect of stomata on the rate of transpiration and also a number of facts and figures are available for the transpiration of the leaf as a whole, very few attempts have been made to ascertain the direct effect of changing external conditions on the mesophyll alone, although it is from these cells that the main water loss occurs. The action of the mesophyll has in most cases never been separated from that of the stomata. The difficulty is to remove the action of the stomata. It was suggested by Knight that leaves from which the epidermis had been stripped might be employed, but the method is drastic, and there is the further danger of the drying of the mesophyll cells except in atmospheres of high humidity.

The other method was that employed by F. Darwin (1914). The essentials of this method are to exclude the action of the stomata by stopping them up with some substance like vaseline and then placing the intercellular spaces in contact with the atmosphere by means of slits in the leaf tissue. Such slits were usually made

with a scalpel between the principal veins of the leaves so as to interfere as little as possible with the water supply. Using this method, Darwin attempted to determine the effects of changes of light and humidity of the atmosphere on the mesophyll tissues of the leaf. He came to the conclusion that the leaf increased its transpiration by amounts that varied between 10 to 100 per cent. of that in the dark. An increase of the latter order is certainly most remarkably high. To bring about such a doubling of the evaporation rate from a moist surface in air of constant evaporating power would require an increase of temperature of at least 10° C. From a careful consideration of Darwin's data, Henderson (1926) was able to ascertain two possible sources of error. Firstly, Darwin's observations were usually begun immediately after smearing the leaves with vaseline and slitting, thus making no allowance for "wound-shock response," and secondly, external conditions were seemingly quite uncontrolled and largely unknown. The plants were in some cases removed from the window of the laboratory into the dark-room, apparently without accurate observations being made of humidity and temperature.

Henderson, by means of a highly ingenious piece of apparatus, has repeated and extended Darwin's observations. The plants used in these experiments were *Hedera helix* (Darwin's original plant), *Eupatorium adenophorum* and *Aster* (sp.). It was found that the transpiration at first rose to a high value after slitting, and then settled down to a steady rate in the course of an hour or so. The cause of this strange behaviour was not ascertained. It was during this preliminary unsettled period that all Darwin's readings were taken. Since all his experiments were begun in the light and the plants removed to the dark-room when the maximum was reached, it was not surprising that a drop was shown in the transpiration rate in the dark, and it is also of significance in this connection that when the plants were again placed in the light, the second curve nowhere reached the same value as the first. Had Darwin started his experiments in the dark, then in all probability his results would have been reversed.

It was shown by Henderson at the conclusion of his experimental work that light does have a small direct effect upon

transpiration from the mesophyll; the order of increase being from 4 to 5 per cent. of that in the dark. The exact action of the light on the mesophyll is unknown. Henderson advanced the view that it may be due to the presence of unknown biological complexes, and is possibly in the nature of a photo-chemical reaction. It cannot, however, be said that the explanation is of a very helpful nature. In any case this action of the light shows that transpiration is not solely a simple process of evaporation. It is possible that a secretion of water from the mesophyll cells of the leaf takes place; the cells actively secreting water or some solution on their outer surfaces. Here again, it must be confessed that the word "secretion" in reality only acts as a cloak for our ignorance of the chemical and physical phenomena underlying the whole process.

The Importance of Transpiration to the Plant.—Transpiration exists in all land plants. Whether it be an advantage or a disadvantage, the fact remains that it is quite unavoidable. The absorption of carbon dioxide from the air by the leaf is a prime necessity for the synthesis of carbohydrates by the living plant, and it follows that there must be some suitable mechanism of gaseous exchange in the organisation of the plant body, and this exchange is principally performed by the stomata of the leaf. As a corollary, it follows that if stomata are present, water losses must occur. The aerial portion of the plant soma is covered with cuticle, and were this cuticle to extend over the whole surface of the plant, it would be unable to carry out its normal function of carbon dioxide absorption and, of necessity, its metabolic activities would cease.

A large number of teleological explanations have been advanced which were considered as proof that transpiration is vital to the well-being of the plant. The best that can be said for these is that they are explanations. It has been supposed that in plants exposed to extreme conditions of heat, transpiration cuts down excess of temperature. This is possibly true within limits. It certainly does not apply to succulents which transpire but slowly, and possess a number of somatic modifications to prevent excessive transpiration. It has also been shown in a number of succulents

that the temperature of the mesophyll tissues is very much higher than that of the surrounding air :—

| Air Temperature | | | | | 28.1° C. |
|--------------------------|--------------------|---|---|---|----------|
| <i>Sempervivum album</i> | (temp. of tissues) | . | . | . | 52.0° |
| <i>Opuntia</i> | („ „) | . | . | . | 43.0° |
| <i>Gentiana cruciata</i> | („ „) | . | . | . | 35.0° |

In the case of ordinary mesophytes the temperature of the leaves is not much higher than that of the surrounding atmosphere, usually about a degree or so. In high winds the temperature may even fall below that of the air. Even when leaves are cut the temperature only rises one or two degrees from “wound-shock.” It must also be remembered that in the case of the ordinary type of dorsiventral leaf with its flat surface, there is a very large area exposed to the air which would tend to prevent any rise in temperature should external conditions of drought and heat arise.

The most exact work on this subject has been carried out by Clum (1926) using leaves of *Fuchsia speciosa*, *Brassica oleracea* and *Syringa vulgaris*. The temperature of the leaves was determined by means of thermo-couples under a number of experimental conditions. The leaves were always found to be at a higher temperature than that of the surrounding air. The effect of shading was found to cause a sudden drop in the temperature; further the direct effect of sunlight was very much marked, and the particular angle at which the rays struck the leaf had an effect on its temperature. In no case, however, could he find any correlation between leaf temperature and transpiration.

A second claim that has been put forward to show that transpiration is an advantage to the plant is that it rids it of excess water and allows of the rapid absorption of salts. A considerable amount of evidence was at one time forthcoming in support of this view, but it has since been shown to be erroneous. The

process of transpiration has nothing to do with the absorption of salts; the two processes work independently of one another. The absorption of salts is a question of the salt and water equilibrium of the cell, and is not connected with the evaporation of water from the leaves. Moreover, the concentration of salts in the plant is very much higher than the concentration of the corresponding salts in the soil. An early experiment of Hasselbring (1914) bears on this problem. He grew tobacco seedlings in the open where transpiration would be normal, and a second series under calico to reduce transpiration. At the end of a given time the ash-content of the two sets was determined, and was found to be identical in both series. The ash of the shade series was 11.2 per cent., compared with 9.2 per cent. for those grown in the open. The shade plants had absorbed 35 litres of water and the sun plants 46 litres or 30 per cent. more. The amount of mineral matter absorbed by the plants was therefore not proportional to the transpiration, nor did the plants with the lower transpiration suffer with regard to salt absorption. Later experiments by other investigators have given substantially the same results. Muenschner (1922) grew barley in water culture, and, by suitable arrangements, was able to cut down the transpiration rate. He ascertained that there was no difference in the ash-content of the series grown under conditions which reduced transpiration; the ash-content could only be correlated with the growth of the plants. Mendiola (1922) found that in tobacco a reduction in the amount of transpiration led to an increase in the dry-weight and a decrease in the ash-content. On the other hand, his results support the view that there is no proportionality between the amount of transpiration and the amount of salt absorbed by the plant.

It is quite possible, however, that transpiration might increase the rate of transport of salts from one part of the plant to another, once the salts had effected an entrance. The rate of the transpiration stream is rapid, while the passage of salts from cell to cell is slow. It has been shown that salts are present in the xylem, and it may well be that the transpiration stream conveys salts from the lower to the upper regions of the plant. There may be a

critical rate of the transpiration stream below which the water fails to carry the salts in the xylem with any rapidity.

The Transpiration Current.—The question of how water is conveyed from the roots to the leaves of plants has long exercised the minds of plant physiologists, and a number of theories have been advanced to account for the process. In some plants, such as the Blue Gums of Australia, which are often as much as 300 feet high, the question of water transport becomes a serious one.

It has been conclusively shown that the path of the current is through the xylem. It is easy to demonstrate this fact by simple experiments. If a cut shoot be placed in a weak solution of such a dye as eosin, the dye will only be found to be present in the tracheæ. Again, a ringed tree will continue to transpire for a considerable time, while water plants which transpire but little, have only weakly developed woody tissues.

There is need here of a dynamic and not a static explanation of the process; in other words, an explanation that will adequately account for the continuous drive of water up the cavities of the xylem. Such phenomena as evaporation, capillarity, atmospheric pressure, and root pressure have all been invoked to account for the ascent of sap. Capillarity and atmospheric pressure are both static processes; after water has risen a certain distance, no further rise can occur. Root pressure is a dynamic process, but it, however, is low in summer, when transpiration is at its maximum, so that root pressure will not adequately account for the ascent of sap in plants. It is, therefore, not surprising that the older investigators fell back on vital theories to account for the rise of the water current, and assumed that some kind of pumping action was initiated by the living cells in the xylem, such as the xylem parenchyma. Strasburger's classical experiments conducted many years ago, showed quite conclusively that water would still ascend in plant stems when the living cells had been killed, and thereby sounded the death knell to any vital theories, and physiologists were forced to fall back on purely physical explanations.

The Cohesion Theory of the Ascent of Sap.—The theory originally propounded by Dixon and Joly in the 'nineties, that the ascent of sap was due to the cohesion of the water columns in the cavities

of the xylem and was drawn off from above by the leaf, has been very fully dealt with by Dixon in his monographs—*Transpiration and the Ascent of Sap*, and the *Transpiration Stream*, and will not be considered in detail here.

The theory Dixon has put forward and supported by a considerable amount of quantitative and qualitative data, has rather tended to overshadow all other work on the matter. The chief disadvantage of the theory is that it explains too much.

Briefly stated, the Dixon and Joly cohesion theory accounts for the ascent of sap by taking into consideration the fact that water possesses strong cohesive properties and that quite considerable pressures may be developed in this way, in fact, pressures as high as 300 atmospheres (*cf.* Renner, 1915) have been recorded. According to Dixon, then, the ascent of sap takes place as follows: the water in the conducting tracheæ hangs in fine columns cohering to the walls of the tracheæ. (From this initial condition we can proceed to a consideration of the passage of water from the soil through root and stem to the leaves.) Through the suction pressure of the root-hairs and cortical tissue, water is removed from the soil and passes into the xylem (see Chapter I.). By means of its cohesive properties the water adheres to the wall of the cavities of the xylem in fine columns, and from these columns, which are in a high state of tensile strain, it is withdrawn by the mesophyll tissues of the leaf. These hanging columns of water can exert a pull equal to that of a fine steel wire, but they can only do this while they are attached to the walls of the containing tracheæ. The initial force in the process is exerted by the root.

Although the cohesion theory of the ascent of sap explains in a remarkable manner a large number of facts that have been observed about the transpiration stream, in actual practice it is difficult to devise crucial experiments which would definitely settle the truth or otherwise of the theory. From the Dixon point of view the matter is reversible, that is to say, the plant should react equally well if it were placed upside down. It must be remembered however, that it would be almost impossible to obtain the same contact between the particles of the soil and the leaves as exists between the root-hairs and the soil.

The main criticism that has been levelled at the theory is the presence of air-bubbles in the wood. The presence of air-bubbles immediately destroys the stability of the hanging columns and would cause their collapse. It is impossible to state definitely whether the air-bubbles seen in cut sections are not brought there by mechanical manipulation, or whether their presence is natural in the wood of the living plant. The investigations of Bode (1923) seem to indicate that there are no bubbles in the xylem normally, and that even in the last stages of wilting the water-content of the tracheæ is continuous. ✓

Dixon accepted the presence of bubbles in the xylem. The difficulty is to remove them. The suggestion has been made that perhaps root pressure in the spring is sufficiently strong to get rid of them. But when the whole plant is transpiring rapidly, a negative pressure is set up in the wood, and the bubbles are then under a negative pressure; when a positive pressure is introduced they will suffer compression.

A further difficulty that arises from the Dixon point of view is the fact that tracheids should be more efficient than vessels for the conduction of water. In Dixon's own words: "The salient feature of the wood is the subdivision of the waterways by an immense amount of transverse and longitudinal divisions into minute compartments . . . the vessels and tracheids. For the system the function of which is the conducting of water this is evidently the most unexpected configuration. It is true that the partitions are permeable to water; but when a considerable distance has to be traversed the sum of the distances opposed by the walls becomes very important. . . . It is evident from the persistence of walls in the development of the water-conduits of a plant, introducing as they do an immense resistance to flow, is inexplicable on any view that regards the water being forced through the stem by some unknown force. The cohesion theory, on the other hand, gives a ready explanation, for it confers stability on the tensilely stressed transpiration/stream." From this statement it is evident that tracheids with their numerous short compartments would give greater stability to a tensilely stressed stream of water than vessels in which the cross-partitions are

relatively far apart. Yet, such being the case, it is surely a curious anomaly that our present dominant flora, the Angiosperms, have practically without exception adopted vessels in place of tracheids.

The various objections raised in this discussion to the unqualified acceptance of the cohesion theory, as Professor V. H. Blackman once remarked: "Somewhat dilute the milk of the cohesion theory." It must be admitted that at the present time there is no adequate explanation of the ascent of sap, and although the cohesion of water possibly plays a considerable part in the matter, it is probably only a portion of the whole truth.

Vital Theory of Ascent of Sap

In spite of the work of Strasburger and others, the vital theory of the ascent of sap has apparently still a hold on some minds. At the present time the most ardent exponent of such a theory is the Indian investigator, Sir Jagadis Chundra Bose. According to this worker, water is driven through the plant by some kind of pulsatory activity of the living cells, these cells being identified with the innermost cells of the cortex. The methods of reasoning which lead him to make these assertions are remarkable and peculiar.

In his book, *The Physiology of the Ascent of Sap*, Bose reviews the various theories that have been put forward from time to time to account for the rise of sap in plants and any view that the rise depends on purely physical grounds is considered to be quite untenable. The work of Dixon and Joly is summarily dismissed in three or four lines, Strasburger is held to have misinterpreted his results (see below), and Bose then quotes a statement from Pfeffer's text-book of plant physiology written over forty years ago (1882) wherein it is stated:—

"How and by what means the water is so rapidly transmitted even to the summit of the tallest trees has not yet been satisfactorily explained. It has unfortunately not yet been determined whether the aid of living cells is quite necessary."

Apparently all that has since been discovered is of no conse-

quence to Bose, who sets forth his views backed by a series of experiments chiefly interesting for the highly complex apparatus that was employed.

According to Bose, a pulsatory activity is initiated in the roots, affecting the absorption of water from the soil. A similar activity in the cortex pumps water up through the stem from cell to cell and also injects water into the vascular tissue of the xylem along which it is physically transferred. The water conveyed by physiological conduction and physical convection reaches the leaf, where it is distributed along the veins and their numerous ramifications, and is eventually excreted and transpired into the intercellular spaces, whence it finds its way to the atmosphere outside by evaporation.

To establish this standpoint, Bose has taken the following experimental line. The pulvinus of *Desmodium gyrans* executes alternate contractions, the period of each pulsation varies according to Bose from one minute to four minutes, in other words, rhythmical activity is strongly developed in certain tissues. Similarly, by means of his "crescograph," Bose asserted that growing organs also exhibit an autonomous activity of a pulsatory nature. Having convinced himself of the truth of these two statements, Bose further stated that the ascent of sap is modified in the same way by different conditions such as anæsthetics. Such being the case, he had no difficulty in formulating his theory that the ascent of sap is effected by the rhythmic activity of living cells. "It should be noted," as Dixon has pointed out in his admirable criticism of this work *The Transpiration Stream* (1924), "the peculiar verbal transitions which often form an essential part of his (Bose's) arguments. Here alternate contractions, varying from one to four minutes or so, become 'pulsations,' and these glide easily into 'rhythmic activity.'"

An experiment by Bose in support of his views is quoted here. He employed an electrometric method depending on his idea that "the electric condition of the tissue undergoes a definite variation under changes of turgor; a diminution of turgor introduces an electric change to galvanometric negativity, while an increase in turgor, on the other hand, an electric change to galvanometric

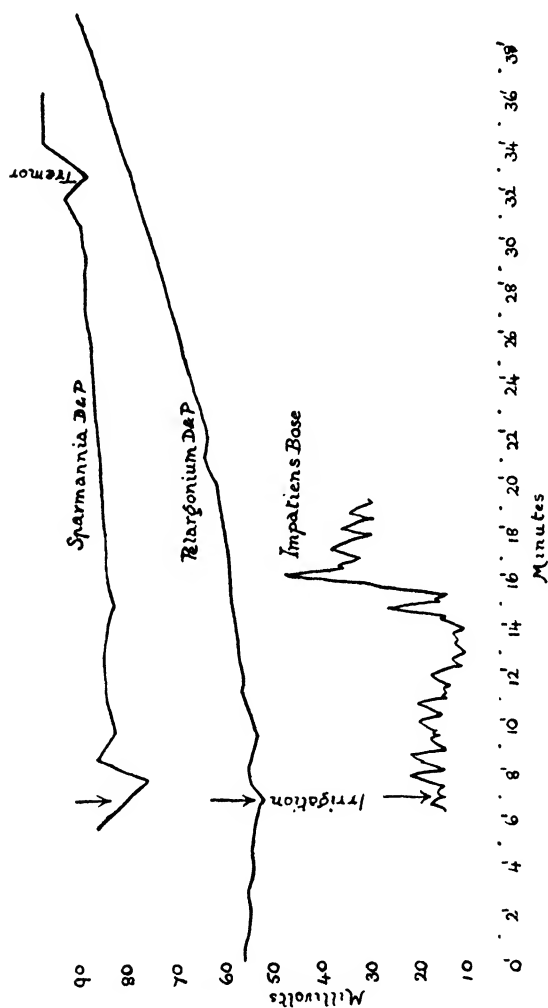


FIG. 22.—Curves obtained by Dixon and Poole (D. & P.) compared with the curve obtained by Bose. For full description see text. (After Dixon, *The Transpiration Stream*.)

positivity." Now a plant under drought has its turgor diminished throughout its length. If then, two electrical contacts are made, A with the stem and B with a distant leaf, the electric condition of the two points will be more or less similar. Addition of water

to the soil, however, will cause an ascent of sap, which will reach the lower contact first and will induce an increase of turgor at this point. This will at once be signalled by a sudden change to positivity at A. This experiment was repeated by Dixon, aided by Dr. J. H. Poole (1924), using a galvanometer which gave a deflection of 1 mm. for 0.004 volt. The plants employed were *Sparmannia africana* and *Pelargonium zonale*. Although the soil round each was dry, neither showed any signs of wilting. "After connection had been made as described by Bose, a slow movement of the spot of light was recorded. A few minutes later the spot came to rest at 19.7 mm. to the left of zero (Fig. 22). This, as the instrument was arranged, indicated that the point A was positive relative to B. When observation showed that the spot was sensibly stationary, the earth in which the plant (*S. africana*) was growing was irrigated with water at about 25° C. Immediately the spot moved to 17.0 mm. and slowly crept back to 19.3 mm., which it reached two minutes later. During the next thirty minutes it crept slowly to 21.0 mm. During all this time nothing like the to and fro motion, corresponding to Bose's pulsations, was observed, nor was there any sudden deflection. Point A was 3 cm. above the surface of the soil. The temperature of the room was 14.0° C." Similar results were obtained for *Pelargonium zonale*. Dixon also failed to confirm Bose's statement of a rhythmic change of potential in the cells of the stem by means of Bose's method of the electric probe. This "electric probe" consisted of a platinum wire coated with celluloid and enclosed in a glass tube. This glass tube was fixed firmly in the body of a microscope, so that the sharp point of the wire, from which the celluloid had been stripped, projected from the glass tube and occupied the position of the objective. With this instrument Dixon attempted to repeat Bose's results. The body of the microscope was placed horizontally and the vertical stem of the experimental plant was firmly clamped to the stage. In this way the point of the probe was gradually advanced through the tissues of the stem by the action of the micrometer screw of the microscope. By means of mercury in the ensheathing glass tube, connection was made between the probe and one pole of a galvano-

meter, while the other pole was connected with another platinum contact embedded in the tissue of a morbid leaf. The circuit was completed through the plant. With this apparatus and using a highly sensitive galvanometer giving a deflection of 1 mm. for a current of 10^{-10} amperes, Bose recorded that the galvanometer showed alternate deflections in opposite directions when the probe had advanced a certain distance inwards. The period of this alternation varied according to circumstances from 13.5 seconds to about three minutes. The amplitude of the vibrations varied between 10 mm. and 66 mm. Dixon, with a galvanometer giving a deflection of 1 mm. for a current of 5×10^{-10} amperes, and using *Pelargonium zonale*, *Impatiens sultani* and *Chrysanthemum sinense*, found that there was a slight "kick" every time the probe was advanced 0.1 mm. into the tissue at intervals of ten minutes, but in no case was there any regular vibration recorded.

Bose considered that the osmotic activities of the leaf cells are too slow to account for the rapid rise of water in plant stems. "He quite fails to realise that slow osmotic activities spread over the large area of the bundle terminations would cause a comparatively rapid rise of water" (Dixon). Bose's criticisms of Strasburger's work are entirely beside the point. He stated that the living cells above the dead portions of the stem are able to pump water through the vessels, and thus "unconsciously admits the substance of the cohesion theory" (Dixon).

Benedict has determined the applicability of Bose's theory by comparing the maximum rate of flow which is possible according to his theory with the known rate of flow. The cross-sectional area of the living cells in the trunks of ten trees of different species was measured, and the maximum pumping capacity at the most rapid pulsation rate found by Bose, was computed. The actual rate of sap flow under maximum conditions of transpiration proved to be from eight to thirty thousand times as rapid as would be possible under Bose's theory, *Quercus rubra* giving the first value and *Robinia pseudacacia* the second, while the other eight species used presented an intermediate condition.

Translocation.—In the higher plants the food material elaborated during active metabolism must be transferred from one part of

the plant to another for purposes of storage. Thus, sugars synthesised in the leaves are removed to other parts of the plant body and stored as insoluble polysaccharide for future use. Similarly, proteins have also to be translocated away. Again salts from the soil solution must be passed up through the plant for a variety of anabolic purposes. It follows, therefore, that paths of passage must exist in the somatic organisation of the plant, so that this process of translocation may be facilitated.

It is important to bear in mind that translocation of material is regulated by supply. Thus substances present in sufficient quantity may undergo very rapid translocation until the needs of the plant are satisfied. Moreover, different substances may be translocated in different directions at one and the same time. In the germinating seed, the reserve stores of food in cotyledon or endosperm have to be conveyed in two different directions: (1) to the upwardly developing shoot, and (2) to the downwardly developing root.

In the case of annual plants growth ceases towards the end of the season and translocation of elaborated food material becomes a serious problem, inasmuch as the ripening fruit and seeds must be supplied with sufficient reserve stores for successful germination in the following season. In annual plants, then, there is an upward translocation to maturing fruit and seeds, while in perennials the reverse holds good, for the elaborated food is passed down to the underground rhizomes, roots, bulbs and similar organs for storage during the winter months of the year. They are utilised from this source when growth recommences in the spring. Translocation is now in the reverse direction, and the stored food, after it is brought into a suitable state for removal by the action of hydrolysing enzymes, is carried upwards into the newly developing buds.

In the higher plants the internal tissues may be roughly divided into wood or xylem which is dead tissue, and the bast or phloem as well as ground parenchyma, which are living cells with protoplasmic contents. The question arises which of these tissues carries away the elaborated food material from the active centres of synthesis.

The older investigators considered that the function of the xylem was to carry water and dissolved salts, while the phloem furnished the channel for the downward passage of the elaborated products of metabolism. The chief evidence for the phloem being the channel of transport of the already synthesised organic food material was largely based on ringing experiments. It has long been known that if a stem were "ringed," then the passage of food apparently ceased. The main line of translocation was considered to be through the sieve tubes; the companion-cells and phloem parenchyma merely played a subsidiary part. Thus the products of photosynthesis and protein synthesis were primarily mobilised in the leaf and were then removed to the storage organs *viâ* the sieve tubes.

The early work of Czapek appeared to give complete confirmation of this picture translocation and his experiments are too well known to need more than the very briefest reference here. Czapek, for example, showed that if the petioles of leaves were killed, either with steam or with chloroform, the depletion of carbohydrates was completely stayed, or if the petioles were cut on one side there was considerable delay in the removal of carbohydrates in that half of the leaf. On the other hand, if the petioles were immersed in a 5 per cent. solution of potassium nitrate, carbohydrate transport was not interfered with. He therefore concluded that plasmolysis of the phloem did not interfere with the transport of sugars. He considered the sieve tubes to be the chief organs of transport because the deposition of callus synchronised with the stoppage of translocation.

It was not until 1911 that any serious criticism was brought against this work. In that year Deleano stated that if the petioles of leaves were killed, even in such circumstances, carbohydrate still left the leaf, although there was considerable reduction in the rate.

Dixon and Ball (1922) seized upon the criticisms advanced by Deleano, and concluded that the upward as well as the downward transport of organic material was through the wood; a view further elaborated by Dixon in 1922 in his Presidential Address to the British Association (Section K).

Dixon, in the course of his address, pointed out the well-known fact that Fischer had found reducing sugars as well as proteins to be present in the xylem, and further that he (Dixon) and Atkins had shown that the sugars in the wood were not only hexoses, but that disaccharides such as sucrose and maltose were also present, and that these carbohydrates were to be found in the tracheæ at all times of the year. Dixon considered that ringing experiments are of little value, inasmuch as the surface of the wood may be injured and more important still, may become blocked with air-bubbles into which substances are exuded by morbid changes in the cambium, medullary rays and wood parenchyma.

However, the more cogent of Dixon's arguments in this connection lie in certain calculations that he has made with regard to the actual quantities of organic material involved in transport and the velocity of flow in the channels which are necessary to effect this transport. He gives as an example a selected potato tuber of weight 210 gm. The total cross-section of the bast in the slender branch was 0.0042 cm.²—no allowance having been made for the thickness of the cell walls. In 100 days all the material in the tuber must have passed through this cross-section. On analysis it was found that 24 per cent. of the tuber was combustible, therefore more than 50 per cent. of carbohydrate passed through this conduit. Assuming the average concentration of carbohydrate to be 10 per cent., the volume necessary to convey 50 gm. must be 500 c.c., and its velocity must have been :—

$$\frac{500}{0.0042 \times 100 \times 24}$$

i.e., nearly 50 cm. per hour. As a matter of fact, the concentration is probably never higher than 5 per cent. and possibly not more than 4 per cent. Dixon considered that the nature of the sieve tubes with their thick, viscous contents, do not admit of such a rapid rate of flow. He further thought that there is a downward as well as an upward flow of water in the xylem and cited two experiments in support of this view. A petiole of *Sambucus niger* was split longitudinally and one half removed and the attached portion was then placed in a solution of eosin. It was found that the solution

was rapidly drawn up the wood capillaries of the intact half petiole and soon appeared in the veins of the pinnæ on the same side of the leaf, beginning with the lowest and gradually working up into the upper ones, and finally into the terminal pinna. In the next stage the dye worked its way through the veins of the pinnæ on the other side, and eventually to the lowest pinnæ on the split side of the petiole. The second experiment of Dixon consisted in cutting the tip of an upper leaf of a potato plant under a solution of eosin. The liquid was quickly drawn back into the tracheæ of the leaf and passed down the stem even into the tuber.

Both experiments, however, are open to criticism. In the case of the leaf of *Sambucus niger* it would be expected under the experimental conditions employed that the dye would be drawn up one series of pinnæ and would eventually pass down the other side. This, therefore, is no proof of the reversal of the transpiration stream. That a backward suck was exerted by the potato leaf is also not surprising if Dixon's cohesion theory is correct. One of the main points of the cohesion theory is the fact that the water is considered to be under a strong tensile strain. In such circumstances, if the strain at one point were broken in any way, the strain under which the water is suffering in the other tracheæ would tend to cause a suck back and show apparent reversal where in reality none existed.

According to Dixon, translocation can be best explained on the cohesion theory of the ascent of sap. "Transpiration, by drawing off water from the tracheæ, causes water filling these tracheæ to pass into a state of tension. This tension determines a flow from any source wherever situated and the continuous column in one series of tracheæ to draw down solution in a neighbouring filament of tracheæ terminating above in some local supply."

Although Dixon's view that the xylem and not the phloem was the channel of transport was accepted for some time after its announcement, there nevertheless has been a growing opposition to the theory, and the most recent work on the subject shows quite definitely that it is the phloem which is the channel of transport of carbohydrates. Arndt (1929) has reported that there is an upward as well as a downward movement of eosin solution in the same

ring of xylem in the coffee tree. The solution usually moves in the outer ring of the xylem. One-sided staining, discovered when the eosin was applied to the lateral roots, indicates that specific roots are connected with particular branches of the crown. In daylight there is a more rapid upward current of eosin, and it is only in the late evening, at night and the early morning, that the downward movement is not greatly reduced. Arndt considered that this downward movement is quite adequate to account for the downward transfer of solutes.

Curtis (1920, 1923, 1925) found that in ringed stems protected with paraffin wax there was a considerable interruption of the upward supply of carbohydrates as well as nitrogen and ash to the growing regions of plants, whereas only very small amounts of xylem were necessary for the supply of water.

By the use of divided stems whereby water was supplied to the top by one set of roots and nitrogen by another, Curtis was able to show that the nitrogen was not transferred by the xylem, while if the xylem were connected by a short strip of phloem then a normal supply would be translocated upwards. The experiment, however, was not of a very satisfactory nature, and by a later series of experiments he was able to show that the xylem alone was not satisfactory as a channel of transport for nitrogen, while the phloem alone was quite as satisfactory as xylem and phloem together.

Dixon's calculations based on the potato tuber is certainly a difficult point to explain, but as Curtis showed, no sugars have ever been found in the xylem of the potato, and further, measurements based on the work of Artschweger indicate that the cross-section of the xylem is smaller than the cross-section of the phloem of the tuber. Curtis agreed with the view put forward by de Vries many years ago that the rotation of the protoplasm helps in the conduction of food down the phloem, and he considered that protoplasmic rotation is not a pathological phenomenon as some investigators have assumed, and cited the cases of *Nitella* and *Elodea*, where the streaming exhibited by the protoplasm is undoubtedly a normal phenomenon of the cell.

If, according to Curtis, the phloem supplies an efficient

mechanism for the upward supply of carbohydrate, nitrogen and ash, then it is probable that it is just as efficient for the downward transport of metabolic products from the leaf.

Weevers (1923), in order to determine whether the upward transfer of foods during periods of terminal shoot growth takes place through the xylem or the phloem tissues employed ringing experiments on the variegated branches of *Æsculus Hippocastanum* and *Acer Negundo*. The use of branches bearing leaves with no chlorophyll eliminated the possibility of the manufacture of food above a ring, which would occur in normal green leaves, thus obviating the necessity for defoliation or for darkening of this part. Ring wounds were protected with wet bandages or the application of cocoa butter. Experiments were carried out in the spring just before the buds started growth and also in midsummer. The experiments consisted of the complete ringing of green and yellow shoots and the partial ringing of yellow shoots. In either spring or midsummer, complete ringing of the shoots prevented normal growth, while partial ringing of the yellow shoots or complete ringing of normal green shoots allowed considerable growth to take place. Analysis of *Æsculus Hippocastanum* showed 3 per cent. of reducing sugars in the green leaves, 1 per cent. in the yellow from unringed branches and only traces from the ringed branches. When ringed, the leaves from the yellow branches often showed early withering and death. Though no variegated branches were available, comparable experiments were conducted with *Nerium Oleander*, which bears phloem strands internal to the secondary xylem. Ringed branches, darkened by enclosure with blackened paper, continued growth indicating that ringing in this case had not prevented food movement. This work shows very clearly that removal of the phloem, contrary to Dixon's statements, does very seriously interfere with the translocation of carbohydrate.

The most conclusive work on this subject has recently been carried out by Mason and Maskell (1928) in Trinidad on the cotton plant. This investigation based entirely on quantitative data can only be briefly dealt with here. For further details the original two lengthy memoirs should be consulted, which are

admirable examples of lucidity of expression combined with care in the interpretation of results.

Mason and Maskell estimated the quantity of carbohydrate in the bark, leaves and wood of the cotton plant at different times of the day. They expressed their results as "residual dry-

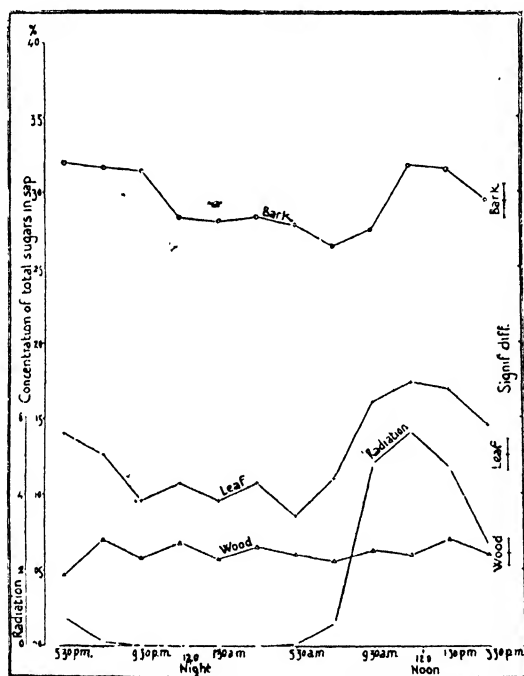


FIG. 23.—Graphs showing the concentrations of total sugars in bark, wood and leaves of the cotton plant. Changes in the sugar content of the bark is very similar to that in the leaf, but tends to lag behind the latter. (After Mason and Maskell, *Ann. Bot.*)

weight," i.e., total dry-weight less carbohydrates. It was discovered that the greatest changes in amount of carbohydrates occurred in the bark and leaves, whereas in the xylem it remained more or less constant in amount (Fig. 23).

Stems were also ringed and the exposed portions protected with vaseline to prevent external changes. It was found that there

was an interruption of carbohydrate supply across the gap. The ringing, however, did not interfere with the passage of carbohydrate into the stem immediately above or immediately below the ring. An accumulation of sugars was found in both bark and wood just above the ring and also in the leaves 2 feet away. Yet throughout all these experiments the concentration of sugars in wood remained fairly constant. Further experiments with ringed stems in which the flaps of bark were separated from the wood by means of paraffined paper, showed that conduction took place at practically the normal rate. Hence connection between bark and wood is not necessary for normal conduction.

Diurnal estimations of the sucrose supply to the bolls showed that translocation was four times as fast by day as by night. The variations of sugar transport into the bolls could be significantly correlated with variations in the sucrose gradient from bark to boll, and it is perhaps by means of these gradients of concentration that the supply of sugars is removed from one part of the plant to another. Samples of bark taken from different levels of the same stems showed that there was a concentration gradient in the bark down the stem towards the roots which resembled the movement of diffusion in that the direction of movement was from a region of high concentration to one of low concentration. The major part of the total fluctuations of sugars in the bark is due to sucrose, and it is therefore suggested that the bulk of the carbohydrate travels in this form.

The main significant difference between physical diffusion of sugar in water and the movement of sugar in the plant is that this movement is enormously greater in the latter. It is, in fact, forty thousand times as great as the diffusion constant in a 2 per cent. solution of sucrose in water, and is almost identical with the diffusion constant for molecules the size of sugar molecules diffusing in air.

Although Mason and Maskell use the term "bark" for the channel of transport, it must be understood that this term covers all the tissues outside the xylem. The main flow of the carbohydrate was discovered to be in the sieve tubes. The question therefore arises as to whether any mechanism in the sieve tubes

can be visualised which would reduce the resistance by the medium to diffusion. It is tempting in the circumstances to postulate the existence in the tubes of some special organisation of capillary structure which greatly reduces any resistance to diffusion. Nevertheless, the simple fact remains that we are quite ignorant of any such structure. A probable mechanism, at least from the physical standpoint, consists in the circulation of the contents of the sieve tubes ; a view, as has already been mentioned, advocated by Curtis. A chain of sieve tubes would then form a series of vortices and exchange of solute between vortices would follow the concentration gradient between the vortices. All the characteristics of the phenomena of diffusion, except the absolute magnitude of the rates, would be shown by this system, so that it would satisfy the known facts with regard to the movement of sugars in the plant. It is evident that there are two aspects of this mechanism. There is first the movement within the sieve tubes, and secondly the movement from sieve tube to sieve tube.

It is suggested by Mason and Maskell that the former might be brought about by the streaming of the contents of the sieve tubes. That is to say, that the concentration within the sieve tubes is rapidly equalised by a mass movement of the contents. The difficulty is that such a movement of the contents of the sieve tubes has not yet been observed, for the streaming of the protoplasm apparently ceases in the mature tubes. It seems also that the rate of this movement, if it should occur, would have to be greatly in excess of the rate of protoplasmic streaming observed in other vegetable cells.

Curtis (1929) has found that when the petioles of *Phaseolus vulgaris* are chilled between 1° C. and 4° to 6° C., the removal of carbohydrate from the leaf blades is greatly retarded or even completely stops. The temperatures which cause a check in translocation are approximately the same as those causing a cessation of protoplasmic streaming, a fact giving additional support to the theory that protoplasmic streaming is intimately connected with solute passage in the phloem. | Chilling also interferes with the upward passage of inorganic material, and if the petioles are enclosed in tubes containing nitrogen under slight pressure, trans-

location ceases. On the other hand, removal of carbohydrate from the leaves does not appear to be appreciably interfered with when the petioles are coated with wax. This may be due to the presence of a petiolar cavity which still allows of effective aeration. This work indicates that upward as well as downward translocation of solutes, organic and inorganic, is brought about by the activity of living cells.

Whatever the actual mechanism may be, the fact remains that Mason and Maskell have demonstrated beyond all doubt that the

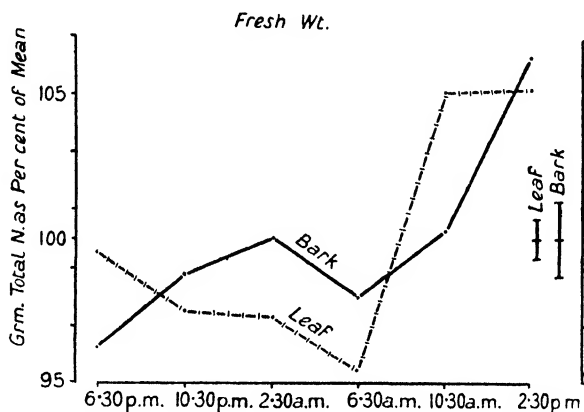


FIG. 24.—Diurnal variations in the total nitrogen-content of leaves and bark of the cotton plant. Values based on fresh weight. (After Maskell and Mason, *Ann. Bot.*)

transport of carbohydrate is *viâ* the phloem, and not as Dixon considered through the xylem.

Although the path of carbohydrate transport in the plant has been so successfully traced by Mason and Maskell, the problem of nitrogen transport is still in an uncertain state. The usual view on the subject is that nitrate from the soil is absorbed by the roots and is moved upwards in the transpiration stream through the xylem to the leaves, where it is synthesised to amino-acids and proteins. The elaborated products are exported down the stem *viâ* the phloem. The whole problem, however, is complicated by the investigations of Curtis (see above), who claimed that not

only is organic nitrogen transported by the phloem, but also mineral nitrogen.

According to Chibnall (1923), there is a diurnal variation in the nitrogen-content of the cabbage leaf, the total nitrogen increasing by day and diminishing by night. It may therefore be concluded from this work that there is a synthesis of nitrogen products in the leaf by day and export of nitrogen by night.

Maskell and Mason (1929) reinvestigated the whole problem of nitrogen transport in the cotton plant. The results were expressed both as fresh weight (*cf.* Chibnall, 1922, 1923) and also as residual dry weight (see above). The nitrogen was estimated in the leaves, bark and wood at different times in the day and night. Expressed on a fresh weight basis, the diurnal variation in nitrogen-content of the leaf followed a definite pattern (Fig. 24). Statistically considered, the variation in nitrogen-content of the leaf showed a definite variation with time, whereas, in the case of the bark, the observed standard deviation due to sampling was not much greater than the standard deviation in time. Therefore, statistically, there was no real evidence of variation in time in the nitrogen-content of the bark tissues. Inspection of the curve suggests that the changes in the bark tend to lag behind those in the leaf. It is possible that concentration changes in the leaf lead to similar changes in the concentration of diffusible nitrogenous substances in the bark sap, and that consequently physical diffusion may be a factor in the transport of organic nitrogen from the leaf. }

Expressed as residual dry weight, the results are not so marked as in the case of the fresh weight values (Fig. 25). Nevertheless, the night values are less than the day values by both methods, and indicate withdrawal of nitrogen by night. When free bolling is taking place the nitrogen-content of the bark just below the foliage region shows no appreciable diurnal variation. This is possibly due to the fact that the bulk of the nitrogen passes into the bolls and little to the roots.

Ringing the stems causes an accumulation of nitrogen in both bark and wood above a ring, and decrease in both below a ring. There also appears to be a horizontal movement of nitrogen from bark to wood. Estimation of nitrate nitrogen shows that ringing

does not prevent its entry into the leaf. Moreover, the downward movement of nitrogen is not interfered with by the separation of the bark from the wood.

A consideration of the data as a whole presented by these authors appears to be in accord with the original view of nitrogen transport, that is to say that the bulk of the mineral nitrogen absorbed by the roots is transported up the stem in the transpira-

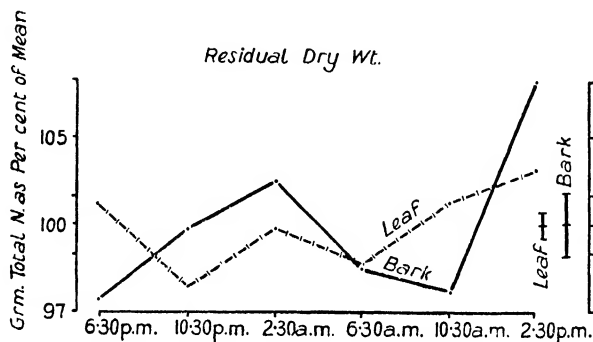


FIG. 25.—Diurnal variations in the total nitrogen-content of leaves and bark of the cotton plant. Values based on residual dry weight. (After Maskell and Mason, *Ann. Bot.*)

tion current through the wood, while the elaborated nitrogen is removed from the centres of synthesis *viâ* the phloem.)

The Passage of Stimuli in Plants.—The reaction of plants to stimuli appears to be connected with certain specific substances of the nature of hormones. Hormones were first postulated by Bayliss and Starling a number of years ago to account for definite animal reactions and were brilliantly verified since, by the discovery and isolation of some of these bodies, such as thyroxin, insulin and adrenalin. They appear to play a rôle in the physiological activities of plants.

The best known case of hormone activity and the one that has been most fully investigated, is that of the sensitive plant, *Mimosa pudica*. The leaves are very sensitive, and close on touching in a very characteristic manner. It was first shown by Ricca (1916) that the stimulus or hormone in *M. pudica* passes through the

xylem and not through the phloem according to Haberlandt. In the animal economy there are two methods whereby a stimulus may be perceived: (1) through the nerves, which form a rapid method of signalling and can be likened to a telegraphic system; and (2) the stimulus may be transferred by a hormone. The latter is a slower method compared with the former, and may be likened to a postal system. In plants, no structures comparable to nerves have been discovered, and although it is equally true that no hormone has as yet been isolated from a plant, nevertheless, from their responses, the presence of hormones must be inferred.

In *Mimosa*, it was shown by Ricca that if the stem were killed with steam or separated by a water-gap, the hormone still passed in the upward or downward direction. This work was further extended by Snow (1924, 1925A), who confirmed Ricca's original observations and added several of his own. He showed that though the phloem of the stem was quite insensitive to the passage of the hormone, the hormone was conducted through the phloem in the petiole, the so-called "high-speed conduction." Although the hormone is thermostable in the plant, watery extracts are killed by boiling. Snow prepared water extracts of the hormone, and found that it was not precipitated by lead acetate and that it gave no protein reactions. It was able to diffuse through a collodion thimble without losing its properties. It is possible that it may have a comparatively simple chemical constitution. Could it be obtained in sufficient quantity a study of its chemical properties would be of the utmost interest.

Ball (1927) has also investigated the conduction of the stimulus in *Mimosa*, and has demonstrated that at least two methods are in existence. The normal method of conduction through the xylem, such as was described by Ricca and Snow, in which the stimulus travels at the rate of about 15 to 28 cm. per minute, was confirmed, and a second still more rapid method of conduction was found to exist in the pith, where the rate of passage is 200 cm. per minute. This latter rapid conduction method is shown when cut shoots are completely submerged in water. Ball proved quite clearly that the stimulus did not pass through the phloem, for on

decortication and removal of all tissues external to the wood, the stimulus still passed with the same rapidity as before. Under normal conditions the hormone is released at the point at which the stimulus is applied and is transported by the water current through the xylem, and this method of conduction operates best when the turgor of the cells is low and the water tension in the vessels is high. When the turgor of the tissues is high, the hormone is still released at the point of stimulation, but, instead of passing up the xylem, it merely causes contraction of the neighbouring cells which are possibly situated in the pith. These in turn liberate a further quantity of hormone, and so a relay mechanism of a highly efficient nature is set up, whereby the stimulus can pass in either direction in the plant and is independent of the water current. Ball has also ascertained that both methods of conduction can operate at the same time. The latter method of conduction (*i.e.*, through the pith) is probably of the same nature as the "high-speed conduction" described by Snow, due to the almost explosive contraction of highly turgid cells; the chief difference lies in the fact that high-speed conduction is through the phloem of the petiole.

The passage of phototropic and geotropic stimuli has recently been shown to be probably due to the transference of hormones. The coleoptile of grasses has been considerably investigated with respect to its response to light, and a very large body of data has now been accumulated on the subject.

As far back as 1832 de Candolle put forward the suggestion that the bending of an etiolated shoot towards incident light was caused by the fact that the highly illuminated side grew less than the side away from the light and a curvature towards the light was thus developed. In other words, the light had a direct effect on the plant, and was directly responsible for the resulting curvature. On the other hand, Pfeffer considered that light acted as a releasing mechanism and there was no quantitative relationship between the externally supplied energy and the resultant response of the plant. The stimulus, in this instance light, acted like the trigger of a gun. There is no quantitative connection between the pulling of the trigger and the ensuing explosion. It

does not matter whether the trigger be pulled quickly or slowly, hardly or softly, the force of the explosion is always the same, and depends on the amount of powder in the cartridge.

This conception of a stimulus affords the best explanation of the results of Charles Darwin with the etiolated coleoptile of various grasses. Darwin discovered that the response of an etiolated coleoptile depends on the perception of the stimulus (in this case light) by a localised region of the organ *which is not the region of curvature*. This work has been confirmed again and again by numerous investigators. The curvature is always present at the base, if the tip of the coleoptile be illuminated. Hence there must be a perceptive and a responsive region. As a matter of fact, it has been discovered that only about 2 mm. of the apical region is sensitive. It is therefore evident that the excitation is released at the tip and travels down to the motor region at the base where it produces curvature.]

Morphologically, the coleoptile is a cylindrical structure with two lateral veins. Rothert (1896) cut the veins and discovered that the stimulus still passed when the tip was illuminated. This observation was confirmed by Fitting (1907). Boysen-Jensen (1910, 1913), however, obtained a different result. He ascertained that if in the air of the laboratory, the vein away from the light were nicked, there was no response. Similarly, if the vein towards the light were cut there was a positive response, *i.e.*, the coleoptile bent towards the light. He confirmed the result of Rothert and Fitting that if the coleoptile were kept in a saturated atmosphere and the vein away from the light were cut, there was a positive response, and the same result was obtained if the vein were cut under water. Boysen-Jensen therefore concluded that the saturated atmosphere kept the gap full of sap and the substance or substances responsible for the response diffusing down from the apex could pass through the water-filled gap. If a mica plate were inserted in the cut made away from the light, there was no response, while if the mica plate were inserted on the side towards the light, curvature still occurred. These results have been amply confirmed by Purdie (1921) and Nielson (1924).

Boysen-Jensen next cut off a centimetre of the tip of the

coleoptile and replaced it by gelatin base. He discovered that on illumination the coleoptile still responded. It is thus evident that the substance responsible for curvature can diffuse through gelatin. This experiment has also been independently confirmed (see Paal, 1917-19; Snow, 1924; Söding, 1925).

Paal has made a number of investigations of coleoptile curvature and considerably modified Boysen-Jensen's original technique. He has shown that none of the results obtained are due to stray light from the illuminated apex. A further experiment of Paal's is important in this connection. He removed the tip of the etiolated coleoptile of *Coix* and replaced it without gelatin in the dark. A curvature occurred through 90° with the side of the stump beneath the tip on the outer convex side of the curve. If, however, the apex were replaced with gelatin, no curvature occurred when the plant was replaced in the dark. Paal concluded from this experiment that there must be two substances or two types of substances diffusing from the tip. One of these promoted growth, but was incapable of diffusing through gelatin, while the second, produced by the stimulus of light, was capable of diffusing through gelatin and was responsible for the curvature.

Boysen-Jensen (1925) considered these substances diffusing from the apex to be growth promoting, and from his experimental results supposed them to pass down in greater quantity on the side *away* from the light. Paal, on the other hand, held that they are perhaps destroyed on the side towards the incident light as a result of illumination.

A series of experiments by Stark (1921) has shown that the apices of foreign genera placed on stumps also give a response. Thus, for example, if the tip of an *Avena* coleoptile were placed on the stump of *Hordeum*, a response was still given, although the reaction was much weaker in the majority of cases. Yet in the particular case quoted here, *Avena* tip or *Hordeum* stump, the response was frequently much stronger than if the decapitated tip of a *Hordeum* coleoptile had been replaced on its own base. Stark also discovered that it did not matter whether the apex were accurately replaced or even if it were replaced back to

front ; the response was always similar. Stark further tried the effect of placing extracts from wounded coleoptiles on the side of the stump and found that response occurred towards that side.

Stark and Dreschel (1922) have also tried the effect of decapitating coleoptiles and illuminating the tips with unilateral illumination. The tips were then replaced on the stumps, and it was found that a response was given in the direction of the original illumination of the tips. An important experiment relating to this work of Stark and Dreschel has been carried out by Brauner (1922, 1924), who removed the tips of the coleoptiles and placed them in the dark and exposed the stumps to unilateral light. The tips were then carefully replaced on the stumps and the plants replaced in the dark for four hours. It was found that twenty-one out of the thirty-two coleoptiles used showed a curvature in the direction of the original light. The control plants, *i.e.*, those in which the tips were not replaced on the illuminated stumps, showed no response at all.

Priestley (1926) objected to the above very positive evidence to prove that the action of the light is to release some substance or substances from the apex, which travel to the base and there bring about a curvature. He considered that since the coleoptile possesses limited growth the curvature exhibited can be explained on simpler grounds. During the period it is employed in phototropic experiments its increase in length is entirely by cell wall extension, no new cells being formed, and since cell wall extension is more rapid in the light than in the dark, though the total increase in length is less, positive curvature occurs.

He recalled the fact that Blaauw (1914, 1915, 1918), with a sporangiophore of *Phycomyces nitens*, equally illuminated by an arrangement of mirrors, the lowest intensity of illumination of which was 210 metre-candle-seconds, showed that no increase in growth occurred for two to four minutes and that this latent period was followed by a marked acceleration in the growth rate. The greater the intensity of the light the less the time of the initial latent period. Yet a sporangiophore submitted to unilateral illumination shows a positive curvature, whereas from Blaauw's results a negative response should have been shown. Blaauw has given

the explanation that this was due to a "lens-action" on the part of the sporangiophore. This structure is cylindrical in cross-section and transparent and Blaauw held that the light was brought to a focus on the side of the sporangiophore away from the light with the result that an acceleration of growth occurred on that side, showing a positive curvature. Priestley therefore considered that there is a greater quantitative relationship between incident light and response which negatives the suggestion of stimulus in one region and response in another. Apical guttation also presents difficulties to Priestley, who stated that the localisation of an apical hydathode explains the direction of the usual autonomous curvature of the coleoptile and he would suggest that the permeability of the coleoptile tissues are increased by light and therefore light falling on the apex will increase apical guttation. Any lateral light that may fall on the apex, therefore, increases the rate of flow from the vein nearer the light and produces phototropic curvature. When the apex is removed guttation is very free and growth much reduced until the cut surface is blocked. If the apex be replaced, the guttation is immediately reduced and growth is greater. If the apex be replaced on the side of the stump only, this side alone is blocked and curvature results. Should the replaced apex have been laterally illuminated, the block is less complete on this side of the stump and positive curvature results.

This theory is too full of a mass of suppositions and assumptions to have any value attached to it. Why precisely a greater flow of sap on the side towards the light (and this is a pure assumption with no experimental foundation) should cause a positive curvature is difficult to say. Priestley's views are still further discredited by the recent important paper by Went (1928), establishing the existence of some growth substance (*Wachstoff*) in the coleoptile of the oat. A number of coleoptiles were grown in darkness and all subsequent manipulations were made upon them in red light which had no effect. The tips were removed and placed upon thin slices of jelly (either gelatin, agar or silicic acid). If a portion of this jelly were replaced asymmetrically on the stump of another coleoptile from which the tip had just been

removed, a negative curvature was found to occur; in other words the coleoptile bent away from the side receiving the jelly. Went interpreted this result as being due to the hormone diffusing from the jelly into the base of the coleoptile. At 25° C. in 170 minutes' time the negative curvature is followed by a positive curvature. This is considered to be due to the regeneration of a new physiological tip, which it would seem is first formed on the side of the decapitated stump away from the jelly, and from here a new supply of growth substances is released into the base of the coleoptile. Went has further shown that the amount of curvature is proportional to the number of tips that were originally placed upon the slice of jelly and to the period of time that they are allowed to remain upon it.

Went suggested that the action of the hormone is to modify the process of extension in cell size. On the other hand, the substance appears to be incapable of extending the size of the cells at the apex of the coleoptile. He therefore suggested that there is a second factor that must be taken into account, namely, materials capable of causing cell wall extension, which must be produced in the base of the coleoptile. Went has made an attempt to identify the mode of transport of the hormone. He found protoplasmic streaming occurring in the cells of the coleoptile and considered this phenomenon to be responsible for the downward transport of the hormone. He has even watched the transference of the substance from a piece of jelly at the top end of a coleoptile stump down the coleoptile to another piece of jelly at the base. Moreover, from rough diffusion experiments he has been able to arrive at the approximate molecular weight of the hormone and found it to be about 376. For further details of this interesting work the original memoir should be consulted. Went's experimental technique is above reproach and leaves no doubt that the response in the coleoptile is due to a hormone and that Priestley's somewhat crude mechanical explanation has no foundation in fact.

It is well known that if the apices of many seedlings of monopodial growth are removed the axillary buds grow out and form new shoots. The apex exerts some influence which prevents

the development of the axillary shoots. The suggestion has been made that an inhibitory substance is formed at the stem apex and is passed down the shoots which prevents the development of axillary buds. Another suggestion is that the growing apex in some way draws to itself the supply of nutrient fluid or perhaps some particular substance necessary for growth, and the axillary buds are thus kept in the dormant condition through starvation. Loeb in a long series of publications on the regeneration of *Bryophyllum calycinum* considered inhibition to be due to the lack of necessary nutrient material.

Snow (1925B, 1929) has critically examined Loeb's work, and considered that the transport of an inhibitory substance or hormone from the stem apex to be the true explanation. Using *Phaseolus multiflorus* and *Vicia faba* he was able to show that ringing the epicotyl, so that all tissue including the cambium was removed external to the wood, still allowed of the passage of the inhibition. The control plants which were decapitated and unringed showed signs of regeneration after four days, whereas the treated plants showed no axillary growth at all. It is thus evident that the inhibition can pass through a stem that has been ringed. He also discovered that the inhibition could pass through the pith, although it is considerably weakened in its effects. When the main apex and axillary bud were only connected by the xylem with a few cells of pith parenchyma adhering to it, inhibition again passed through. In a second series of experiments a zone of the stem of *Vicia faba* was killed by a jet of steam playing on it for twenty to thirty seconds. Although only the outer cortical cells were killed by this treatment the inhibition failed to pass owing to "physiological shock" and the axillary buds grew out below the treated zone, the main stem continuing its growth. When the cut surfaces of tissues of seedlings were bound together in pairs and each pair was decapitated the inhibition was not able to pass across from one to the other. The question as to whether the inhibition could or could not pass across a watery gap proved difficult to show experimentally. The difficulty, however, was surmounted by splitting *Phaseolus* seedlings longitudinally through the lower region of the epicotyl, cotyledons, hypocotyl and main root and

binding the parts together. The inhibition was found to pass through the watery gap.

To test whether the inhibition passed upwards in the transpiration stream, young seedlings of *Vicia faba* were decapitated in the epicotyl, so that the two axillaries of the cotyledons grew out. Those seedlings were then selected in which the axillaries were nearly of equal strength and the shorter of the two was then decapitated above its second leaf and a bud was allowed to remain in the axil of one of the two leaves of this shoot, while the bud in the axil of the other was removed. The remaining bud on the shoot showed scarcely any growth even after three or four weeks. Presumably therefore it was inhibited by the apex of the longer shoot, for if this were also decapitated then the bud grew out strongly. A zone of tissue (about 4 mm. long) was now killed by a hot glass rod without charring the tissues or causing the cell-contents to boil and the dead zone was painted over with vaseline. The controls, in which the apex of the longer shoot was also removed as well as the axillary buds, save the one to be measured, showed a mean growth of $3.37 \text{ mm.} \pm 0.25$ compared with $1.54 \text{ mm.} \pm 0.13$ for the treated plants. The difference of the mean is 1.83, which is 4.36 times its own standard deviation and therefore fully significant.

The interpretation placed by Snow on these experimental results is that an inhibiting substance formed at the growing apex of the longer shoot is transported through the living tissues and leaks into the xylem just below the dead zone. It is then drawn up with the transpiration stream through the dead zone and leaks out into the living tissue of the shorter shoot where it inhibits the growth of the axillary bud.

Root-Pressure.—The excretion of fluid is a common phenomenon throughout the plant world and may take place either from the intact plant or after injury, as in the bleeding of cut stems. This exudation of water falls into two well-marked types, which are termed respectively *bleeding* and *guttation*. In the case of bleeding, it is well known that if the stems of many plants are cut, especially in the spring, there is a copious flow of liquid which is excreted under considerable pressure. This is usually

spoken of as *root-pressure*, but a better term would be *bleeding-pressure*, for although it is undoubtedly true that roots excite the most active exudation of water, this is merely because they are the best absorptive organs the plant possesses. The root-

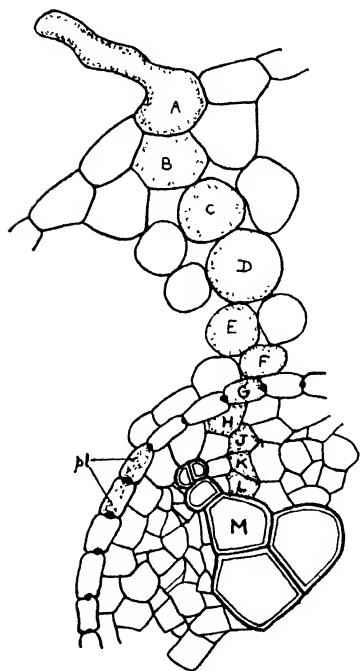


FIG. 26.—Transverse section of a root. A, root-hair; B, C . . . F, cortical cells; G, endodermis showing Casparian strips on radial walls; H, pericycle; M, vessel. (After Priestley, *New Phyt.*)

stock does not, as a matter of fact, always bleed more actively than portions of the stem and in a number of cases it has been found that active bleeding will take place in stems severed from their root-stock. The term “root-pressure” is therefore something of a misnomer in this respect and is better replaced by the term “bleeding-pressure.” The term “guttation” covers the exudation of drops of fluid from the uninjured parts of plants, such as the leaves, hydathodes, nectaries and similar structures.

The fluid that escapes in bleeding may be either practically pure water or may contain considerable quantities of organic matter. The sap of *Acer platanoides* contains 1.15 to 3.4 per cent. of cane sugar, while other plants, such as the vine and potato yield practically pure water. The sap is exuded from the xylem, either tracheids or

vessels. Bleeding, therefore, corresponds with filtration under pressure and the exuding liquid may or may not be mixed with bubbles of air.

Bleeding is a vital phenomenon and is markedly affected by external conditions. A dry soil or active transpiration may cause considerable diminution of bleeding, and anæsthetics such

as chloroform also exert a considerable effect. When the temperature is too low bleeding ceases, although the absorption of water still continues. Finally oxygen is a necessary factor, and in its absence bleeding is brought to a standstill.

-The Mechanism of Exudation.—The exact mechanism of bleeding is still unknown. Priestley (1920) has advanced an explanation based on the fact that roots possess a primary endodermis with Casparian strips on the lateral walls. He stated that osmosis plays a primary part in the process whereby sap is driven up the xylem from the roots. In the accompanying diagram (Fig. 26) which represented a diagrammatic cross-section of a young root he considered a series of cells, A to L, bordering on the xylem vessel, having cellulose walls permeable to water and the majority of solutes, and lined with a layer of cytoplasm which functions as a semi-permeable membrane. Since these cells frequently contain stored starch and presumably, therefore, sugar dissolved in the sap, he contemplated a gradient of sugar concentration from L to A, with the greatest concentration at L and the smallest at A. An osmotic gradient will therefore be set up in these circumstances and water will tend to pass from A with the lowest osmotic pressure towards L with the highest osmotic pressure. ✓ The entering water will gradually distend the parenchymatous cells tightly packed within the vascular cylinder. The difficulty is to consider the passage of the water from L into the vessel M. Unless the water in M contains a sufficient concentration of solutes to be in osmotic equilibrium with L, water may be withdrawn from M to L. The presence of the endodermis is now invoked to force water from L to M. Since the cells H, J, K and L are confined within the endodermis, the extensibility of which is limited, the radial walls being clamped by their thickenings and therefore preventing any lateral leakage, there can be practically no expansion and a sufficiently high hydrostatic pressure will therefore be developed to force water from L to M. This hydrostatic pressure is not sufficient in itself to force water into M. M would have to retain an appreciable osmotic pressure, and since M contains no living contents the sole conditions under which this could become possible would be in circumstances which would

permit of a constant supply of solutes to M from adjacent cells. Priestley considered that the investigations of Lepeschkin on hydathodes offer a ready explanation of the matter. Lepeschkin made the assumption that a different semi-permeability existed on the upper and lower side of the top cells of the sporangiophore of *Pilobolus* to account for the excretion of water by this organ. Priestley therefore assumed that the protoplasmic membrane of L where it abuts on to M is of relatively greater permeability than the protoplasmic layer of L where it abuts on to other parenchymatous cells. In such circumstances water will be forced from L to M, so long as the hydrostatic pressure within the core enclosed by the endodermis is strong enough to force water and solutes into the xylem from L.

When the stream of water and solutes has entered the xylem the only direction in which it is free to move is upwards in the cavity of the vessel. The difficulty of the entrance of the solutes into the xylem and the exit in many cases of pure water, is explained by the assumption, that the solutes are adsorbed on the protoplast surfaces lining the xylem vessel, and this physical adsorption is followed by physiological absorption, whether the solutes in the ascending stream are organic or inorganic substances.

This theory has been submitted to drastic criticism by Professor V. H. Blackman (1921), which has completely robbed it of any value it may have possessed.

As Blackman pointed out, Pfeffer at the time of his classical researches on osmotic pressure, formulated three hypotheses to account for the exudation of fluid from living cells: (a) that the plasma-membrane develops unequal osmotic pressures in different parts of the cell; (b) that there is an unequal distribution of osmotic material in different parts of the cell; (c) that the osmotic material is present in the cell wall outside the membrane so that water is sucked out of the cell. The first hypothesis is obviously unsatisfactory, since it makes the osmotic pressure a function of the membrane instead of the concentration of the solution. Pfeffer himself later abandoned this view. Yet Lepeschkin accepted this first alternative in spite of the fact that Pfeffer had discarded it many years before.

The permeability of the membrane can only indirectly affect the osmotic pressure of the solution it encloses by controlling through exosmosis the concentration of the solution. Any osmotic pressure developed by a leaky membrane of this nature will be dependent in part only on the specific permeability of the membrane. The time during which exosmosis has continued and the original concentration and mass of the solute will be important factors bearing on the subject. If the membrane be rigid, the first pressure developed will be practically the same as if the membrane were truly semi-permeable. "Lepeschkin further holds that not only has a *membrane* a specific osmotic pressure, but that directly this pressure is exceeded water begins to pass out through the membrane. The same solution can thus at the same time be both in equilibrium with and not in equilibrium with water; or rather, that it can be on both sides of the equilibrium point at the same time!" (Blackman). Lepeschkin's views are of little worth in this connection. A further criticism that can be made about Priestley's results is that a gradient of osmotic pressure does not necessarily establish a flow of water from the soil to the tracheæ. This flow of water depends on the suction pressure of the cells, and will therefore also depend on a gradient of suction pressure being established across the cells of the root (see Chapter I.). As Thoday published his paper in 1918, two years before Priestley, it is difficult to understand why the latter failed to mention it. Priestley has suggested that in bleeding the soluble substances which pass into the vessel below are absorbed again during their passage up the stem. But this requires the support of a subsidiary hypothesis, which must seriously weaken any theory. Furthermore, the term "physiological absorption" is a somewhat meaningless phrase,

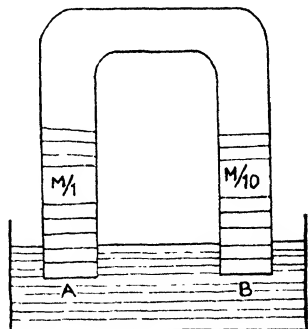


FIG. 27.—Apparatus to illustrate Pfeffer's theory of root pressure. For full description see text. (After V. H. Blackman. *New Phyt.*)

and again, why should cells which give out substances below absorb them again above ?

As Blackman remarks, Pfeffer's original scheme to explain bleeding is more in accordance with the known facts. In this scheme the osmotic substance is supposed to exist in the cell at two different concentrations which is represented by Blackman in the model (Fig. 27). The curved glass tube is closed by two completely semi-permeable membranes, A and B. The arm A is filled with a strong cane sugar solution (say M/1) the arm above B with a weak cane sugar solution (say M/10) the intervening space is filled with water. Water will enter through A and B, a pressure will be developed in the tube, and when this pressure reaches that of the osmotic pressure of the solution above B, absorption above B will stop ; since, owing to the pressure, the solution on one side of the membrane B will be in equilibrium with water on the other side. At A, however, there will be no equilibrium at the two faces of the membrane owing to the higher concentration of the solution, and water will continue to enter. As a result the pressure in the tube will go on increasing, and there will no longer be any equilibrium at B, but water will be forced out and the solution in that arm will become more concentrated. If B were not in water but in air, drops of water would appear at its surface. A transference of water has occurred through the tube, and so work has been done, but there is no contravention of the second law of thermodynamics ; for the process will not continue indefinitely as the two solutions will gradually mix and the work has been done at the expense of the energy of diffusion. This view of Pfeffer's is quite in accordance with the known facts of the matter and does not require any assumptions of a questionable nature.

Priestley and Armstead (1922) claimed that solutes present in the xylem sap are both organic and inorganic. As the organic solvents fall off in quantity in the sap rising from a cut vine, the exudation pressure begins to fail, whilst the supply of inorganic solutes remains practically constant. These investigators therefore considered that organic solutes are more directly responsible for the osmotic pressure in causing the flow of sap. The organic solutes are said to be mainly sugars, both disaccharides and mono-

saccharides. The claim was also made that sugar solutions drawn through lengths of different stems show a fall in sugar concentration, and they considered this to support Priestley's theory of root pressure. It must be confessed that the experimental technique employed in this work leaves much to be desired and adds little if anything to the validity of Priestley's theory.

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CHAPTER III

CARBON ASSIMILATION

Theory of Limiting Factors.—The First Sugar of Photosynthesis—Chemical Mechanism of Photosynthesis.—Chemistry of Chlorophyll.—Photosynthesis and Chlorophyll Content.—Chloroplasts and their origin.

THE whole subject of carbon assimilation has been so recently reviewed in two extensive monographs by Stiles (1925) and Spoehr (1926) that only a few important aspects fall to be recorded here.

One such aspect is the question of "limiting factors" which still gives rise to active discussion. In 1905 F. F. Blackman, in his classic paper on optima and limiting factors, described a scheme to account for the interaction of a number of factors in their effect on the assimilation process which is best stated in the author's own words: "When a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the slowest factor." That is to say, the factor present in lowest concentration limits the rate of the process. To discover which is the limiting factor the following principle is applied: "When the magnitude of a function is limited by one set of possible factors, increase of that factor, and that factor alone, will be found to bring about an increase of the magnitude of the function."

The graph demanded by the Blackman theory was a straight line showing a sharp break when the limiting factor came into operation (Fig. 28). Such graphs were obtained by Matthaei (1904), Blackman and Smith (1911), and Wilmott (1921), and the validity of the theory was accepted without question for over a decade.

Hooker (1917) and Brown (1918) both submitted the theory to a good deal of destructive criticism. Brown considered that

the curve obtained by Blackman and Smith for the assimilation rate of *Elodea* at two different light intensities, was in reality

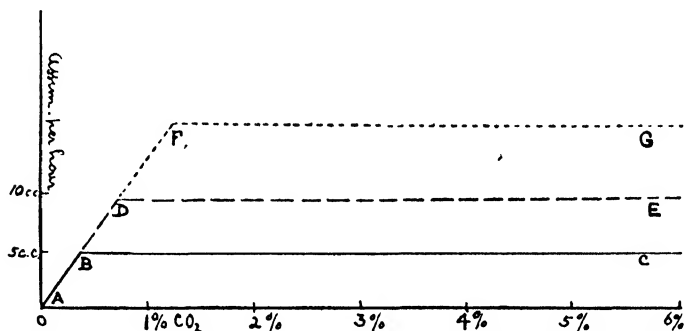


FIG. 28.—Curves illustrating the theory of “limiting factors.” (After F. F. Blackman, *Annals. Bot.*)

made up of two separate curves, and that no sharp break existed. There was, therefore, no operation of limiting factors. The whole

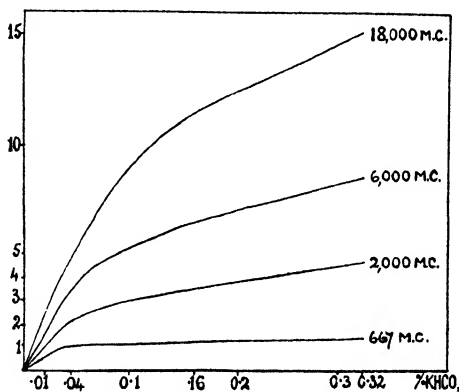


FIG. 29.—The rate of assimilation in different concentrations of potassium bicarbonate and constant light intensity. (After Harder.)

question revolves round this fact : is there or is there not a sharp break in the curve, or is the curve logarithmic in form ?

Boysen-Jensen (1918), using *Sinapis alba*, found a smooth curve

to exist, and no sharp break was obtained when one of the factors became limiting in the Blackman sense.

The most important investigations on this subject were conducted by Harder (1921, 1923). The material used was *Fontinalis antipyretica*, *Cinclidotus aquatilis* and two species of *Cladophora*. The plants were allowed to assimilate in solutions of potassium bicarbonate of different concentrations, and the rates of assimilation were measured by estimations of the amounts of oxygen evolved by a volumetric method. Harder was able to show that the curves obtained in all cases were logarithmic in shape and

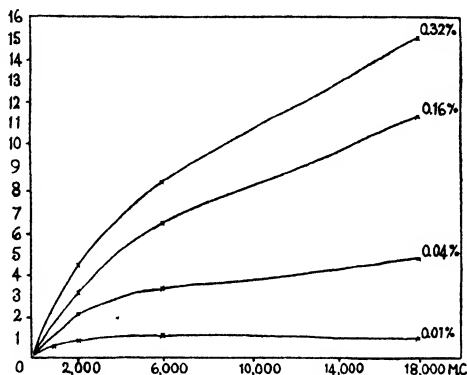


FIG. 30.—The rate of assimilation at different light intensities and constant amounts of potassium bicarbonate. (After Harder.)

exhibited no sharp break. The matter was tested in two ways: (1) all the factors, save one, were kept constant; and (2) variations in two factors were followed. The curves obtained are shown in Figs. 29 and 30. Harder pointed out that in the course of these investigations the same material was always used, whereas Blackman employed fresh lots for each experiment, and the points of his curves lie on such an irregular line that they might be either continuous or show a sharp break. A consideration of the second series of experiments conducted by Harder, in which two factors were varied together, show that an increase in either factor brings about an increase in the assimilation rate. It is obvious that the factors are mutually interdependent and that the

relationship between the two is of a complex nature, and, according to Harder, it is the factor which is in relative minimum which is most important.

Warburg (1919, 1920), using a modification of the Haldane-Barcroft method of gas analysis and the unicellular alga, *Chlorella*, obtained similar curves to Harder. He ascertained that in low concentrations of carbon dioxide, the assimilation rate was

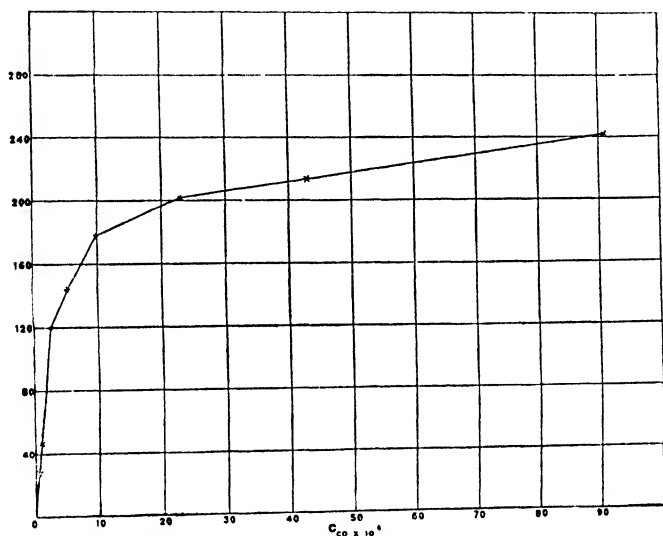


FIG. 31.—The rate of photosynthesis at different concentrations of carbon dioxide. The ordinate represents the rate of photosynthesis, abscissa the concentration of carbon dioxide. (Reconstructed from data given by Warburg. From Spoehr., *Photosynthesis*.)

directly proportional to the concentration. With progressive increase in the concentration the curve exhibited a continuously smaller increase in the photosynthetic rate until it appeared to be independent of the concentration of the carbon dioxide (Fig. 31). Warburg placed the following interpretation on his results: that the rate of assimilation is proportional (i.) to the concentration of the carbon dioxide, and (ii.) to the concentration of some second substance which reacts with the carbon dioxide.

These investigations of Harder and Warburg have been confirmed by James (1928), who found that moderately high con-

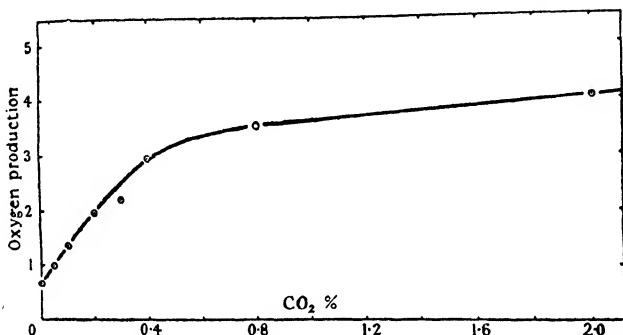


FIG. 32.—Curve of assimilation rate in 0–2.0 per cent. CO_2 at light intensity 20. (After James, *Proc. Roy. Soc. Lond.*)

centrations of bicarbonate or carbon dioxide solution depressed the assimilation rate of *Fontinalis antipyretica*. With a low light

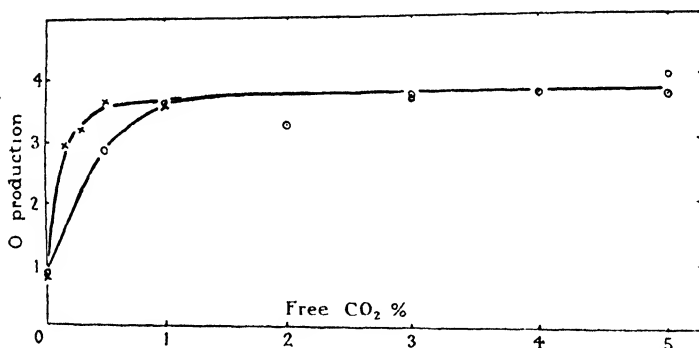


FIG. 33.—Rate of assimilation in solutions of 0–5.0 per cent. CO_2 moved through the apparatus at 400 and 600 c.c. per hour. Series \odot = 400 c.c. per hour. Series X = 600 c.c. per hour. (After James, *Proc. Roy. Soc. Lond.*)

intensity of 20 (arbitrary units) and at a temperature of 19°C . and low concentrations of carbon dioxide (0.05 to 2.00 per cent.), the curves obtained took the form of an oblique hyperbola (Fig. 32).

In higher concentrations of carbon dioxide (0.5 to 5.00 per cent.) and low light intensity (20), it was found that the rate of assimilation was independent of the concentration of carbon dioxide over the range 3 to 5 per cent. carbon dioxide (Fig. 33). Varying both light intensity and carbon dioxide concentration simultaneously, curves very similar to Harder were obtained, *i.e.*, the factors were interdependent and not independent in their effect on the photosynthetic rate (Fig. 34). In these experiments carbon dioxide

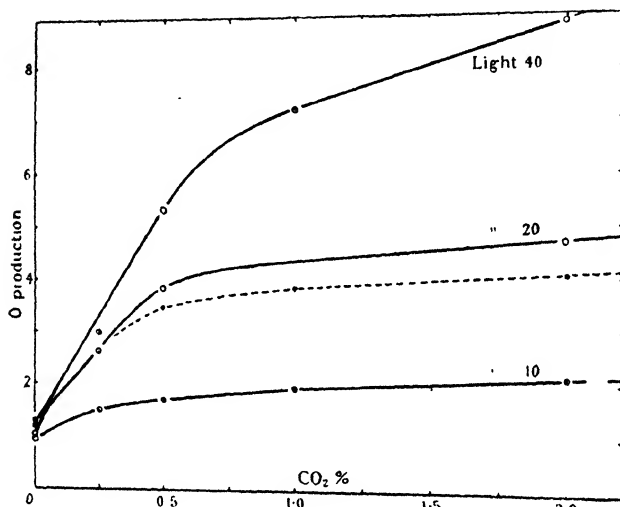


FIG. 34.—Rate of assimilation in CO₂ concentrations 0—2.0 per cent. and different light intensities (10 to 40). (After James, *Proc. Roy. Soc. Lond.*)

was supplied to the plant in solution, and the flow of liquid through the assimilation chamber was at a rate of 400 c.c. per hour. Experiments were also made with sodium bicarbonate as a source of carbon dioxide, and it was discovered that at the same rate of flow per hour (400 c.c.) the sodium bicarbonate gave rise to a higher rate of assimilation than a pure solution of carbon dioxide of equal partial pressure when no other factor was limiting. At a flow of 600 c.c. per hour with low light intensity and consequent slow assimilation, the two solutions gave the same assimilation.

It would seem that in bicarbonate solutions only the free carbon dioxide is available for photosynthesis. At a higher light intensity (80) the bicarbonate again gave rise to a faster rate of assimilation than a pure solution of carbon dioxide of equal partial pressure, though the increase of the rate of flow to 600 c.c. with bicarbonate solution at this light intensity did not increase the rate of assimilation.

James pointed out that the older investigators considered assimilation rate to be almost proportional to the concentration of carbon dioxide at low concentrations, but since a faster flow of liquid tends to make this factor disappear and return at higher light intensities, it is probable that the linearity of the curves obtained was due to the conditions of diffusion obtaining in their experiments rather than to internal stages in photosynthesis. The most acceptable idea, therefore, is to consider the suc-

cessive stages in photosynthesis as being made up of a series of linked reactions, each member of the series being reversible.

The majority of the work on the validity or otherwise of the theory of limiting factors has been carried out on aquatics. Investigation of the problem in land plants is beset with a number of difficulties. It has been shown by Maskell (1928) that variations in the uptake of carbon dioxide by the leaf are due to variations in stomatal resistance and the remaining resistances of the leaf. The stomatal resistance is considered to depend on (1) the season of the year, the range of opening being different in

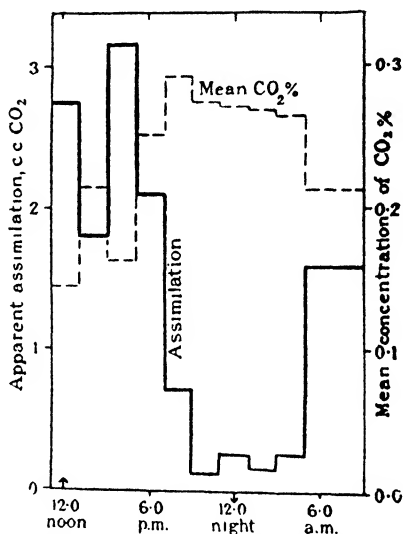


FIG. 35.—March of apparent assimilation compared with mean CO₂ concentration under continuous artificial illumination. (After Maskell, *Proc. Roy. Soc. Lond.*)

different months of the year under conditions of constant light intensity ; (2) time of day—there is a well-marked diurnal rhythm of stomatal opening at constant light intensity ; (3) time elapsing from the beginning of the experiment ; (4) intensity of illumination ; (5) previous history of the leaf ; (6) previous moisture conditions of the plant.

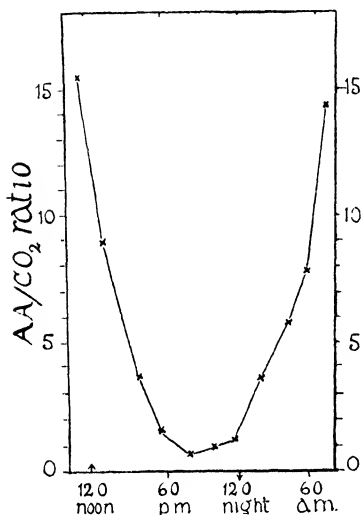


FIG. 36.— March of apparent assimilation in Cherry Laurel leaves under continuous artificial illumination. (After Maskell. Modified, *Proc. Roy. Soc. Lond.*)

Maskell, using *Prunus lauro-cerasus* var. *rotundifolia* and the continuous current method of estimating assimilation, ascertained that there was a diurnal rhythm of photosynthesis with low concentrations of carbon dioxide and a constant intensity of light. The assimilation rate fell during the afternoon and evening, and rose again during the morning (Fig. 35). The apparent assimilation, rather than the real assimilation, was measured, as it was the uptake of carbon dioxide that was principally being investigated. A better picture of the march of assimilation is obtained by calculating for each reading the ratio

between the apparent assimilation and the mean carbon dioxide concentration, *i.e.*, the ratio :—

$$\frac{\text{A.A.}}{\text{CO}_2} = \frac{\text{Apparent Assimilation}}{\text{CO}_2 \text{ concentration}}.$$

Simultaneous observations on the porometer and photosynthetic rate showed these results to be due to the march of stomatal aperture (Fig. 36).

In high concentrations of carbon dioxide, such that light instead of carbon dioxide was limiting the rate of assimilation, no diurnal rhythm was found. Moreover, besides this diurnal

rhythm, a seasonal rhythm was also discovered in the assimilation rate, and Maskell was able to show that the pitch or level of the diurnal photosynthetic curves was higher in July, August and September, than at any other times of the year.

Maskell accepted Warburg's picture (see below) of a photochemical phase in assimilation as a simple expression of a possible type of relationship between carbon dioxide concentration and light at the chloroplast surface. If this be so, then the rate of assimilation is proportional to :—

$$\frac{L \times C_s}{C_s + K_L}$$

where L is the light intensity, C_s is the concentration of carbon dioxide at the chloroplast surface, and K_L is the ratio of the velocity constant for the dissociation of some photochemical product (the rate of formation of which is proportional to L) to the velocity constant for the combination of the photochemical product with carbon dioxide. Expressing the light intensity in terms of light limiting values of the real assimilation (y), then :—

$$y = \frac{L \times C_s}{C_s + K_L}$$

If the resistance to the diffusion path of the respiratory carbon dioxide up to the chloroplast surface be small, or if the path be short compared with the path outside the leaf, then we have :—

$$\text{Assimilation (A)} = \frac{C - C_s}{D} = \frac{L \times C_s}{C_s + K_L} - R$$

where C represents the external concentration and D the total resistance of the leaf to the diffusion path of carbon dioxide from outside the leaf up to the chloroplast surface. Hence the real assimilation (y), is :—

$$y = \frac{L \times C_s}{C_s + K_L} = \frac{C - C_s}{D} + R$$

from which :—

$$y = \frac{C}{D} - \frac{K_L}{D} \frac{y}{(1 - y)} + R$$

and, for the curve relating y to increase, we have :—

$$\frac{dy}{dC} = \frac{1}{D} \left(\frac{1}{1 + \frac{K_L}{D} \left(\frac{L}{(L-y)^2} \right)} \right)$$

when $y = L$, *i.e.*, when the light limited value is recorded, $\frac{dy}{dC} = 0$ or $y = L$ forms the horizontal asymptote to the curve relating y and C . If $y = \pm \infty$, $\frac{dy}{dC} = \frac{1}{D}$, this being the slope of the asymptote to the ascending limb of the curve. Similarly, for the curve relating assimilation and light intensity, we have :—

$$y = L \frac{\frac{C}{D} + R - y}{\frac{C}{D} + R - y + \frac{K_L}{D}}$$

then :—

$$\frac{dy}{dL} = \frac{\frac{C}{D} + R - y}{\frac{C}{D} + R - y + \frac{K_L}{D}}$$

when $y = \frac{C}{D} + R$, then $\frac{dy}{dL} = 0$. This gives the horizontal asymptote of the curve. When $y = \pm \infty$, $\frac{dy}{dL} = 1$. This is the slope of the asymptote to the ascending limb of the curve.

Thus, for any given fraction of the maximal possible assimilation, $\frac{C}{D} + R$, at any given carbon dioxide concentration, $\frac{dy}{dL}$ falls below unity according as D , C or R are relatively small, or K_L is relatively great. That is to say, the corner of the curve relating light and assimilation will be sharp for relatively high concentrations and gradual for relatively low concentrations of D . If this equation forms any approximation to the real relationship between the factors controlling photosynthesis, the divergence found in the forms of curves relating assimilation to carbon dioxide concentration or light intensity represent variations due largely to

different relative values of the resistances in the diffusion and photochemical phases of the process.

The *Elodea* result of Blackman and Smith would correspond to a set of conditions of high resistance to diffusion or high deficiency of light intensity. On the other hand, Harder's results would correspond to low resistance to diffusion or low intensity of light at the chloroplast surface or perhaps to both. Using such expressions, calculated and experimental results obtained by Maskell are given in Fig. 37. It will be seen that the general results obtained

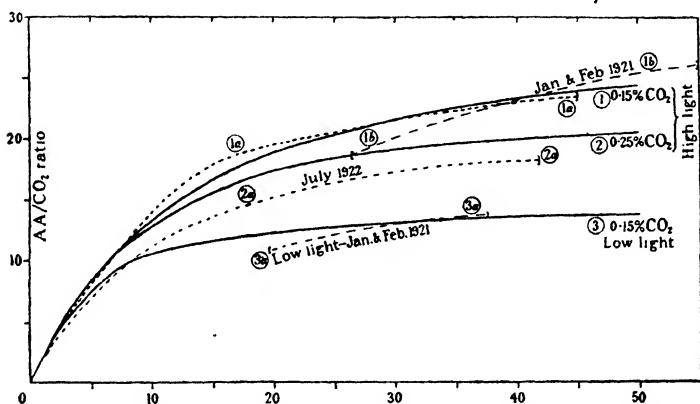


FIG. 37.—Theoretical relation between stomatal diffusive capacity and assimilation (mean CO_2 per cent. and light constant). Theoretical curves (1, 2, 3) are shown by the continuous lines. Experimental curves (1A, 1B, 2A, 3A) by broken lines. (After Maskell, *Proc. Roy. Soc. Lond.*)

from a consideration of the experimental data can be reproduced in the theoretical curves, and that the types of interaction found between apparent assimilation and stomatal opening, seasonal changes in photosynthetic activity and light intensity follow naturally from the theoretical formula.

As Maskell very rightly pointed out, the original conception of the theory of limiting factors was only defined as a first approximation, and has been widely misconstrued as a rigid law "... and the term 'limiting factors' has been borrowed on all hands for the purpose of describing phenomena in *quasi*-quantitative

terms with the minimum amount of quantitative work . . . regarded and used as a clue to the interpretation of phenomena, the general principle of limiting factors as suggested by Blackman in 1905 cannot yet be replaced."

The First Sugar of Photosynthesis

The normal green plant in the presence of light and chlorophyll synthesises carbohydrates from carbon dioxide and water, and thereby converts radiant energy into chemical energy. The question arises which is the first sugar formed. Sachs, in his classical experiments, showed that starch was a product of photosynthesis and demonstrated the fact that it was formed in the light and disappeared in the dark. Sachs described the starch as the "first visible product" of photosynthesis. Starch, however, is not always a product of photosynthesis. It is produced in storage organs and in many plants, especially among the monocotyledons; it is not normally found in the leaves, and only occurs in the guard-cells of the stomata.

The starch molecule from the chemical standpoint is complex, and it is therefore unlikely that it is the first formed carbohydrate of assimilation, and it is probable on this ground alone that simpler sugars like the hexoses and disaccharides precede its formation.

The carbohydrates of the leaf have been submitted to numerous investigations, both qualitative and quantitative. The quantitative investigations of Brown and Morris in 1893 definitely established the fact that sugars were formed in the leaf as a result of assimilation. In the leaves of *Tropæolum majus* they found *d*-glucose, *d*-fructose, sucrose and maltose, but no pentoses. All subsequent investigations have failed to reveal the presence of the *l*-isomerides of these sugars. Davis and Sawyer (1914) claimed to have discovered pentoses to be present in the leaves of the mangold. The presence of pentoses has been denied by other investigators. Their presence was inferred from the fact that furfural was obtained on distillation with 12 per cent. hydrochloric acid (Kröber's method). This method was employed in this

work, but it has been shown to be inaccurate in the presence of other carbohydrates. Further, the presence of uronic acids, *i.e.*, carbohydrate derivatives with carboxyl and aldehydic group in the molecule, also give furfural on distillation with hydrochloric acid, and these substances have a wide distribution in the plant world (see Ling, Paton and Nanji, 1925). Davis, Daish and Sawyer (1916) were unable to confirm Brown and Morris's statement that maltose was present, and they considered that this result may well have been due to the hydrolysis of starch by diastase in the leaf cells as the material was not killed with sufficient rapidity.

Summarising these results up to the present point, it can be definitely stated that the products, either direct or indirect of assimilation are: glucose, fructose, sucrose and starch. The question before us is this: which sugar is produced first in the process? The matter, unfortunately, is still in the controversial stage. Such an investigation is difficult on experimental grounds. The amounts of these substances in the leaf are small, and unless the manipulations concerned with their isolation and estimation are carried out with extreme care they may suffer conversion into other products. Moreover, the interpretation of the experimental data is also beset with difficulties. The rates of change of these substances may be very different, and other complications are caused by translocation and respiration. On purely chemical grounds it would be expected that hexoses should be the first formed carbohydrates of photosynthesis. Yet the major number of workers who have investigated this particular problem of plant physiology have arrived at the conclusion that sucrose is the first sugar produced.

Two possibilities have to be considered in this connection: (a) the so-called upgrade sugars, synthesised from carbon dioxide and water; and (b) the down-grade sugars, produced by the hydrolysis of reserve carbohydrates, such as starch, inulin and other polysaccharides.

Brown and Morris, from their investigations on *Tropaeolum majus*, came to the conclusion that since sucrose was always in excess of glucose and fructose in the leaf, sucrose must be the first

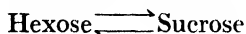
sugar of carbon assimilation. The accumulation of fructose was explained as being due to the greater consumption of glucose in respiration. Excess of sucrose was considered to be converted into starch, while for purposes of translocation sucrose was inverted to glucose and fructose and removed as such. It was found, after assimilation had been allowed to proceed all day, that the leaves contained no glucose and very little fructose, but considerable amounts of cane sugar were present. This was considered to lend further support to the view that sucrose must be the first sugar of photosynthesis. If translocation were prevented by cutting the petioles, then there was an increase in sucrose and starch, but not in glucose and fructose. It must be mentioned here that the methods employed by these workers were not above reproach.

Davis, Daish and Sawyer (1916) considered that the large amounts of maltose found by Brown and Morris were due to the hydrolysis of starch by diastase. Gast (1917), on the other hand, also working with *Tropeolum majus*, in large measure confirmed the findings of Brown and Morris, and also found maltose to be present in the leaves. This latter confirmation was no doubt due to the fact that he employed the same method of killing the leaves, *i.e.*, by heating them to 100° C. in an oven. Gast, however, showed a considerable amount of caution in his final deductions, and did not commit himself to the view that sucrose is the first sugar of assimilation, but called it the "first analytically recognisable sugar." Parkin (1911), working on *Galanthus nivalis*, also came to the conclusion that sucrose is the first formed sugar in the leaf.

The work of Davis, Daish and Sawyer, briefly alluded to above, was an elaborate investigation on the mangold to determine whether sucrose or hexose was the first sugar of photosynthesis. They were able to show that no maltose was present in the leaf, and the proportion of pentose was fairly constant in amount. The quantity of sucrose was always in excess of glucose, and on these grounds they considered that the sucrose was the first product of the assimilation of the leaf.

Although under the very exact analytical conditions employed

by these workers there can be no doubt that sucrose was always found to be in excess of hexose in the leaves, yet it would seem to be a misinterpretation of experimental data to conclude that sucrose is therefore the first sugar of photosynthesis. Chemically speaking, it is very unlikely that such a state of affairs should exist. As V. H. Blackman has pointed out, if formaldehyde be the initial product of carbon assimilation (and there seems little doubt at present that this is the case, see p. 166), it is more probable that the aldehyde should straightway be polymerised to hexose rather than to a disaccharide like sucrose. Blackman therefore suggested that a hexose is first formed and that a state of equilibrium exists between hexose and sucrose :—



when the concentration of the hexose reaches a certain value it is converted into sucrose. Hence the value of the hexose is practically constant in amount, and this is the condition which has been found to exist. If there be a fall in the value of the hexose, then the sucrose is hydrolysed back to hexose, so that the hexose still remains constant in amount.

Priestley (1924) took very much the same standpoint. He considered that since sugars are formed as intermediate steps in the formation of more complex anhydrides, such as starch, it is necessary that these reactions should take place smoothly and rapidly : “ It is to be expected then that the intermediate stages of the process, including the sugars first formed, instead of accumulating in the light and therefore fluctuating in amount, should pass rapidly into other substances in the complex chain of metabolic changes, so that little if any change in their concentration can be detected, and in any case, no such phenomenon would occur as a local accumulation such as is characteristic of a storage product ” (Priestley).

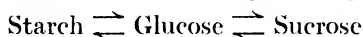
A second contention made by Priestley hardly appears to be in accord with the known facts of the case. He considered that cane sugar is a secondary product in the metabolism of the meristematic cells, being occasionally released from these cells during vacuolation and differentiation. Such an assumption is very doubtful,

and also very unnecessary ; it is much more probable that cane sugar is translocated to these cells as such, and there inverted as required (see Chapter II., under the work of Mason and Maskell).

As Parkin (1925) pointed out, cane sugar is a product peculiar to the vegetable kingdom. It has a wide distribution and is found in every group of green plants. The Phæophyceæ and Rhodophyceæ have still to be investigated in this connection, for although Kylin was unable to detect it in the Phæophyceæ, he discovered the presence of glucose and fructose. It would therefore appear that it should be present. In the mammary gland, glucose previously stored as glycogen travels through the blood stream and is converted into lactose and the following equilibrium exists in the system :—



Parkin therefore suggested that perhaps the equilibrium :—



exists in the plant. Chapman (1925) considered that the system is very much more likely to be :—



and that the reaction velocity is greater in the second reaction than the first. Again, the reverse series of changes should also hold :—



and in this case the reaction velocity of the glucose \rightleftharpoons maltose system would be greater than the maltose \rightleftharpoons starch system.

Ahrms (1924), in his study of wilted leaves, found that they contained more sugar than starch compared with the controls with normal water supply. This is certainly contrary to expectation. It is possible that in wilting the hydrolytic enzymes of the leaves are brought into closer contact with the substrate and hydrolysis takes place more easily. He also found that diminution of starch content gave a proportionate increase of hexose and sucrose combined or *vice versa*.

Weevers (1924) has up to the present time put forward the most

cogent evidence that hexose and not sucrose is the first sugar of photosynthesis. Realising the difficulties of the earlier investigators who all used green leaves, he employed variegated leaves of a number of species instead, and estimated the sugar content of the green and non-green portions. In *Acer Negundo*, *Hedera helix*, *Humulus lupulus*, *Pelargonium zonale* and a number of other plants, he ascertained the presence of both sucrose and hexose in the green portions of the leaves and only sucrose in the non-green parts. Two exceptions to this rule were discovered: *Cornus sanguinea* and *Æsculus Hippocastanum*. Here hexose was also found in the non-green portions, but only in very small amount. *Pelargonium zonale*, when deprived of sugar and allowed to assimilate, gave only hexoses, and this was followed by sucrose, and finally starch. The fact that sucrose alone was found in the non-green parts of the leaf, and that both hexose and cane sugar were found in the green portions, coupled with the further fact that when leaves were allowed to assimilate for a short time only hexoses were formed, points almost irresistibly to the conclusion that hexose and not sucrose is the first sugar of assimilation.

Tottingham, Lepkovsky, Schultz and Link (1926), working with the sugar mangold and sugar beet, found that in the leaf-blade sugars increase with the solar radiation within certain limits. A temperature, for example, of 30° C. acts as a limiting factor on the increased production of reducing sugars in the presence of high illumination. The sucrose content varies very irregularly in the leaves and over a considerable proportion of the period investigated followed the course of the reducing sugars. Occasionally, however, it varied inversely as the hexose content. In view of these results, they considered that hexose and not sucrose is the primary product of the assimilation of the leaf.

If a hexose and not sucrose is the first formed product of photosynthesis, a further difficulty has to be contended with, for not one, but two hexoses, glucose and fructose, must be produced to give cane sugar by condensation. In this connection Nef (1913) showed that aqueous solutions of *d*-glucose, *d*-fructose, and *d*-mannose, in the presence of a 0.05 equivalent of calcium hydroxide at the ordinary temperature, gave mixtures composed of *d*-mannose,

d-glucose, *d*-fructose, *d*-pseudofructose, as well as α -, β -, and *d*-glucose. Similar results have been obtained by Spoehr and Wilbur, who have been able to bring about interconversion of sugars in the presence of solutions of disodium hydrogen phosphate, and in neutral mixtures of this salt and sodium dihydrogen phosphate. Again, in the animal economy, fructose is converted into glucose. On these grounds, if glucose be first produced in the leaf, the mesophyll might be possessed of a special and at present unknown mechanism to convert a part of the glucose into fructose and other hexoses. Nevertheless, mannose has never been isolated as such from the green leaf; its recognition should be simple, as it is the only known hexose which forms a hydrazone.

The whole subject of the first sugar of photosynthesis requires reinvestigation. All the investigations cited above have been conducted under uncontrolled external conditions. What is very urgently required at the present time is a critical reinvestigation conducted under rigidly controlled conditions of temperature, light supply, humidity and concentration of carbon dioxide.

The Chemical Mechanism of Photosynthesis

The chemical mechanism of photosynthesis has led to almost endless speculation and no very convincing proof. It is obvious that the process takes place in stages, and that the first stage is one of reduction. The main question that has to be decided is whether this reduction is brought about by photochemical means, or, if it be due, as Warburg held, to the production of some substance in the cell, which, under the influence of light, is capable of reducing carbon dioxide.

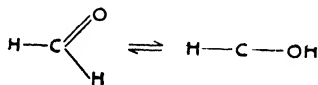
It must be emphasised that carbon assimilation is an endothermic reaction, that is to say, a reaction in which heat is absorbed. The sugars produced, whether hexoses or disaccharides, are compounds of higher potential energy than the initial substances, carbon dioxide and water. If photosynthesis were a purely photochemical reaction, then the Van't Hoff coefficient, Q_{10} , would be less than 2.0. Matthaei, it will be remembered, found the value to be approximately 2.0. It follows, therefore, that some other

purely chemical reaction or possibly even some enzymic reaction is involved as well.

The main theory of the chemical reactions involved in photosynthesis and the one that seems to have fascinated the minds of nearly all subsequent investigators, is that put forward as far back as 1870 by the German chemist, Baeyer. Struck by Butlerow's experiments on the action of calcium hydroxide on solutions of formaldehyde in which a complex mixture of sugars were produced, Baeyer suggested that the first product formed in assimilation from carbon dioxide and water was formaldehyde, and that this was subsequently polymerised to sugars. The theory has been tested by innumerable investigators, and it must be confessed that the majority have pronounced in its favour on no very adequate grounds.

The most recent support for Baeyer's hypothesis emanates from the laboratory of Professor E. C. C. Baly, who with his co-workers has published a number of papers on the subject which will be considered here.

In the first communication, Baly, Heilbron and Barker (1921), pointed out the well-known fact that carbon dioxide absorbs light of wave-length $200\ \mu\mu$, while formaldehyde absorbs light of a wave-length $290\ \mu\mu$. Hence the suggestion was put forward that if photosynthesis were to take place, it would occur in two stages: (1) the formation of the aldehyde from carbon dioxide and water, which would require light of wave-length $200\ \mu\mu$; and (2) the subsequent polymerisation of this aldehyde, which would require light of wave-length $290\ \mu\mu$. These workers considered that ordinary formaldehyde, which is a highly toxic substance to the plant, is not produced, but the tautomeric form of the aldehyde is formed in the leaf:



and it is this "active" aldehyde that is polymerised to reducing sugars.

This early work of Baly and his co-workers led to a storm of

criticism. Spoehr (1923) was quite unable to find any evidence of the production of formaldehyde from carbon dioxide and water on illumination with a quartz mercury vapour lamp as Baly laid claim to have done. Baly replied that Spoehr must have used the wrong shape of lamp! Irving and Francis (1924) examined the mixture obtained by Baly, on exposure of solutions of formaldehyde to ultra-violet light, and stated that the syrup appeared to contain 25 per cent. of sugar (calculated as glucose), but the amount was reduced when the solution was heated with acid. Methylation appeared to show that 9.3 per cent. of sugar was present in the mixture, while the bulk of the material was definitely a non-sugar in nature, but contained hydroxyl groups. Porter and Ramsperger (1925) reported that illuminated solutions of carbon dioxide form formaldehyde when they come into contact with sealing wax, cork or rubber, whereas, if the entire apparatus be constructed of quartz, no aldehyde is produced.

The very active criticism produced by his work has made Baly shift his original position very considerably, and he now holds that it is unnecessary to postulate two separate phases for photosynthesis, and that the activated aldehyde produced from carbon dioxide and water can polymerise itself to reducing sugars without loss of energy and subsequent reactivation.

According to Baly, Davis, Johnston and Shanassy (1927), when an aqueous solution of carbon dioxide is exposed to ultra-violet light, a so-called photostationary state is produced. Ordinary formaldehyde is not a component of this system of equilibrium, but there is present a component which is probably a complex aldehyde. They found that ferrous bicarbonate in aqueous solution in the presence of ultra-violet light and in the absence of oxygen was converted into ferric hydroxide with the simultaneous formation of organic compounds with reducing properties. This reaction appeared to take place on the surface of the quartz tubes employed in the experiment. They further found that when insoluble barium sulphate and metallic aluminium were suspended in water through which a stream of carbon dioxide was passing, and the whole was exposed to ultra-violet light, complex organic compounds of the nature of carbohydrates were produced. In

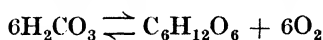
still further experiments on this subject, Baly, Stevens and Hood (1927) discovered that carbon dioxide adsorbed on the surface of either nickel or cobalt carbonate suspended in water photosynthesised organic compounds in the presence of ordinary light. The amount of photosynthesis in the presence of coloured catalysts was greater than in the presence of white powders like barium sulphate. On this ground, therefore, Baly and Davis (1927) considered that there is a marked similarity between photosynthesis *in vivo* and *in vitro*, and that the following features appear to be common to both :—

1. Ordinary formaldehyde does not take part in either.

2. The laboratory process is realised by the action of carbonic acid on a surface. In the plant it takes place on the surface of the chloroplasts.

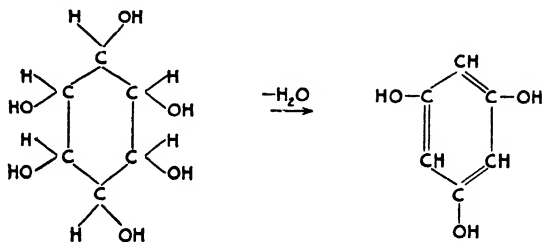
3. A visibly coloured surface and visible light function in both.

Baly's latest view, therefore, appears to be that the theory as originally advanced was wrong. The mistake lay in supposing that light of two different wave-lengths was necessary in the process. He considered this to be quite unnecessary, and that there is only one stage. The carbonic acid absorbs light and becomes activated, at the same time losing oxygen to give active aldehyde ($\text{H}-\text{C}-\text{OH}$). A photostationary state occurs when pure carbon dioxide is exposed to ultra-violet light :—

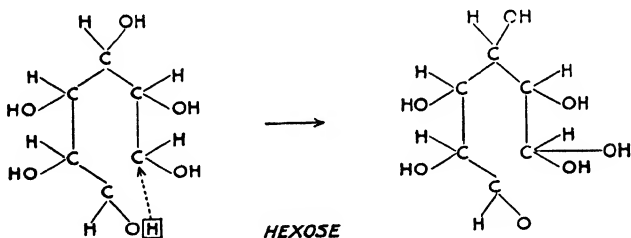


This photostationary state is considered to explain the results obtained by Porter and Ramsperger with apparatus entirely constructed of quartz. Any kind of reducing substance present will take up the oxygen, for example, iron or aluminium, and suffer oxidation. Again, with coloured compounds such as the salts of nickel and cobalt, this reaction will take place in ordinary light on the surface of the catalysts. In the plant, the carbon dioxide is supposed to combine with the surface of the chloroplasts, and the action of the chlorophyll is considered to be analogous with that of the coloured catalysts nickel and cobalt carbonate. It is the agent which causes the transformation of the active region of the light from the ultra-violet to the visible.

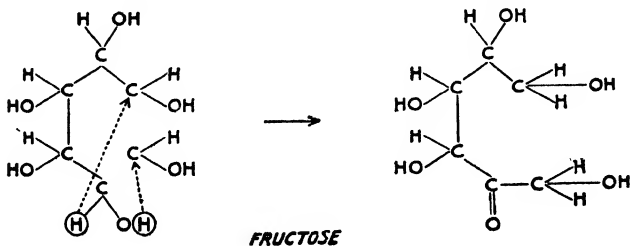
With regard to the polymerisation of the active aldehyde, ($\text{H}-\text{C}-\text{OH}$), the carbon will tend to realise two desires : (1) to become tetravalent, and (2) to take the most stable configuration. If polymerisation give a six-ringed compound, both desires will be simultaneously realised and satisfied :—

*PHLOROGLUCINOL*

A further possibility is the wandering of one of the hydrogen atoms of a hydroxyl group to an adjacent carbon atom with the breaking of the bond between the two carbon atoms :—



This is the constitution of a hexose. If we imagine two hydrogen atoms to wander, then the result would be fructose :—



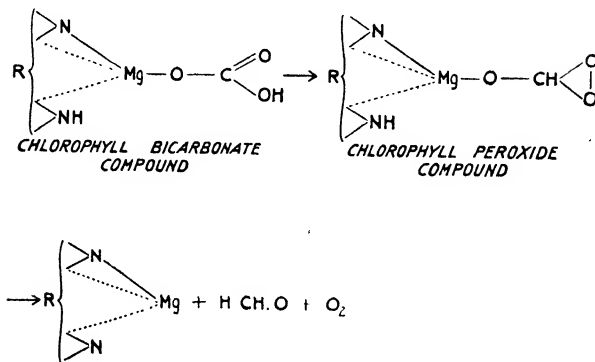
This, however, is a suggestion with no experimental proof.

Baly and Hood (1929) claimed that temperature has the same effect on their *in vitro* experiments as in the living cell. They determined the yield of carbohydrate at different temperatures, using nickel carbonate as a catalyst for the reaction. A linear relation was found to hold between 5° C. and 31° C., and there was a rapid decrease in yield at higher temperatures than this. Although the relationship is not quite linear in the case of the leaf, they attributed this to the following causes: the simultaneous production of protein, and the orientation of the chloroplast to light may be different at higher temperatures.

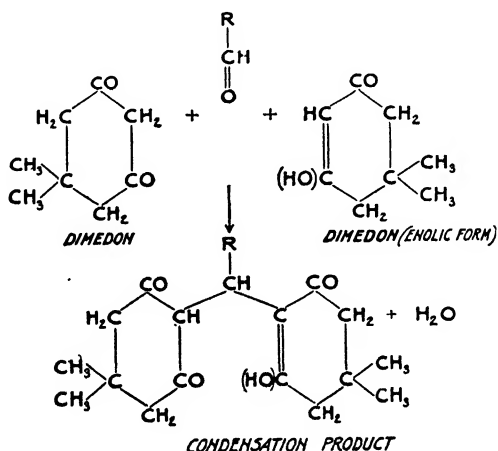
It must be remembered that the whole of Baly's work has been conducted by means of *in vitro* experiments, and the plant has never been considered. It cannot be too strongly emphasised that the plant is not a test-tube. Because certain reactions take place in a certain way in the laboratory, it does not follow in the least that they can take place in that particular manner in the living cell. The chemical reactions of the mesophyll tissues of the leaf are complex in the extreme, and it is somewhat absurd to compare the rough-and-tumble heroics of Baly's test-tube experiments with the marked smoothness of the processes of the photosynthetic mechanism of the plant. Moreover, the numerous modifications that he has been forced from time to time to introduce into his original conception of the chemical mechanism of carbon assimilation, and the manner in which he has been dislodged from one untenable position only to take up another, allows one to place but little, if any, faith in the investigations of this worker.

Willstätter and Stoll (1918) considered that at least four reactions are involved in the formation of sugars from carbon dioxide and water. From experiments on living leaves and leaf powder, they came to the conclusion that chlorophyll is in a colloidal state in the chloroplasts. The Willstätter and Stoll theory also revolves round the formation of formaldehyde as a first product of photosynthesis. They held that carbonic acid is added to the magnesium atom of the chlorophyll molecule. As a result of the absorption of light by the chlorophyll complex, intramolecular change takes place with the formation of a compound of higher energy content; this substance is of the nature of a peroxide. Under the influence

of an enzyme the peroxide undergoes decomposition into chlorophyll, formaldehyde and oxygen :—



The most definite and reliable evidence for the production of formaldehyde from carbon dioxide and water in photosynthesis was found by Klein and Werner (1926) using the new reagent dimedon (dimethyl-cyclo-hexanedione) as a test for this substance. The dimedon reacts with aldehydes to give a condensation product :—



In the case of formaldehyde, formaldimedon is produced, and the crystals of this substance differ in shape from those obtained from

acetaldehyde and dimedon, so that the two can be distinguished. They discovered that the reaction was only shown by leaves containing chlorophyll which had been exposed to light. In the presence of narcotics such as phenylurethane and hydrocyanic acid, no formaldehyde was produced. Should this work be correct, it settles a long-standing controversy.

Warburg's Theory.—Warburg (1919, 1920, 1921, 1923; Warburg and Uyésugi, 1924) has brought forward a theory of the mechanism of photosynthesis which is an improvement on any of those mentioned above, inasmuch that it takes into account the fact that the reaction is taking place in a living organism. He considered that there are several steps in the process, the first of which is the so-called "photochemical primary reaction." In this reaction light is considered to act directly upon chlorophyll with the formation of a "photochemical primary product," the rate of formation of which is proportional to the amount of radiant energy absorbed per unit time. A second reaction now takes place between the photochemical primary product and an "acceptor." This acceptor is not carbonic acid itself, but is formed from the acid by a series of chemical reactions in the cell which are independent of the presence of light.

Warburg drew these conclusions from the fact that in high concentrations of carbon dioxide and high light intensity, the intensity of the assimilation rate does not increase with increase of these factors. Further, in such circumstances, with a rise of temperature of 10°C. the rate of photosynthesis is doubled, *i.e.*, the Van't Hoff law is followed. Warburg called this the "Blackman reaction." On the other hand, at low light intensities, the Van't Hoff coefficient, Q_{10} , is near unity; a result characteristic of photochemical reactions, and shows that light is playing some part in photosynthesis. Thus, with high light intensity, $Q_{10} = 2$, a value characteristic of ordinary chemical reactions, Warburg held that under these conditions a "dark reaction" is controlling the rate of photosynthesis.

Tsi-tung Li (1929) supported Warburg's view. He found an initial inhibition effect on the assimilation rate of a number of water plants when they were changed from light of high available

energy for photosynthesis to one of low available energy. Similarly, there was an initial acceleration effect when the plant was moved from light of low available energy to one of high available energy. Li considered that the splitting off of oxygen does not take place in the primary photochemical reaction, but in a separate later reaction, which is possibly of enzymic nature. The actual degree of correlation between these two reactions varies with individual plants. For example, in a plant with a slow enzymic reaction, the initial inhibition and acceleration effects may be obscured. It is in many ways unfortunate that this author did not employ Wilmott's bubble-counting technique for his work, rather than the haphazard older method of counting the bubbles that arise from a cut stem. The errors in the older method are so numerous that they might conceivably invalidate his conclusions.

Warburg also studied the effect of narcotics on the photosynthetic rate, and discovered that potassium cyanide inhibited the use of the carbon dioxide of the air for photosynthesis. When, however, the light intensity was reduced so that the respiratory rate exceeded that of assimilation, the rate of the latter was unaffected by concentrations of KCN of 0.02 M and under. In these circumstances, the carbon dioxide of the air was not used for photosynthesis, but no carbon dioxide of respiration was released in the light, and, according to Warburg, either this respiratory carbon dioxide or perhaps some intermediate product of respiration is used in assimilation. Again, Warburg found that should a certain concentration of KCN inhibit the rate of photosynthesis by 50 per cent. at a high light intensity, a similar concentration will have no effect at low light intensity. This is taken as showing that the secondary reaction between the photochemical primary product and acceptor is unaffected by KCN, but that the Blackman or dark reaction is the one affected by the narcotic.

These results and views of Warburg have been obtained by the study of the living plant, and are on this account more to be relied upon than *in vitro* experiments. It is probable that the elucidation of the chemical mechanism of photosynthesis, which up to now has been such an elusive problem, will be eventually

solved along lines of work similar to the investigations of this worker.

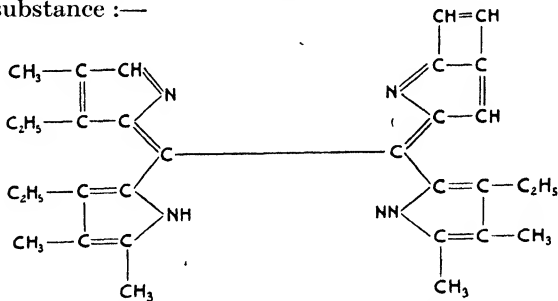
✓ The Chemistry of Chlorophyll

The work of Willstätter and Stoll, carried out over a number of years, showed that the pigments of the chloroplast are composed of four substances: chlorophyll *a*, chlorophyll *b*, carotin and xanthophyll. It is probable that xanthophyll is not a single substance, but a mixture of closely related isomerides and several investigators have claimed to have separated it into different fractions.

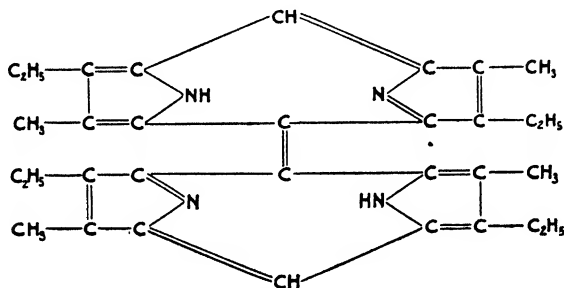
By the action of acids and alkalis, Willstätter and Stoll were able to show that chlorophyll *a*, $C_{55}H_{72}O_5N_4Mg$ and chlorophyll *b*, $C_{55}H_{70}O_6N_4Mg$, were possessed of the same ultimate constitution, and that both gave the same magnesium-free body, *ætioporphyrin*, $C_{31}H_{36}N_4$. The steps whereby this substance was obtained are given in all the larger text-books of plant biochemistry (see Haas and Hill, "The Chemistry of Plant Products," Vol. I., 1928), and will not be considered here.

The ultimate composition of *ætioporphyrin*, however, is of great interest and importance, for it is probable that it is the groups within its molecule which are responsible for the characteristic properties of chlorophyll. It is also of interest on account of its possible relationship to the pigments of the blood.

The structure of *ætioporphyrin* was not established by Willstätter and Stoll, although they were able to show that it contained pyrrole rings, and they suggested the following constitution for this substance:—



The constitution of this substance has now been established by direct synthesis by Fischer and Klarer (1926), who have shown it to possess the following constitution :—



ÆTIOPORPHYRIN (FISCHER & KLARER)

It will be seen that Willstätter's original conception is very near the truth.

Photosynthesis and Chlorophyll-content

Willstätter and Stoll (1918) came to the conclusion that there was no relationship between photosynthesis and the chlorophyll content of leaves. There are a number of inherent difficulties in the use of leaves for an investigation of this nature. The temperature of the cells is never accurately known, and the palisade cells of dark-green leaves tend to screen the internal and lower cells, so that all are not equally illuminated.

This problem has been reinvestigated by Emerson (1929), using the unicellular alga *Chlorella vulgaris* to eliminate the difficulty of equal illumination. By varying the number of cells in a suspension, equality of illumination is readily controlled and the temperature of the cells is not significantly different from that of the surrounding medium which is easily kept constant.

The experiments were carried out at a constant temperature of 19°–21° C., and the illumination used was from a 40-watt lamp. The concentration of carbon dioxide was high, 5 per cent., so that the rate of photosynthesis was independent of small changes in

carbon dioxide concentration. The assimilation was ~~measured~~ manometrically by Warburg's method of estimating the amount of oxygen evolved. Cultures were grown with varying amounts of ferrous sulphate, so that they developed a range of chlorophyll concentration. It was found that the respiration did not vary with the chlorophyll content, and a uniform correction for respiration was made. It was found also that at high light intensities the rate of photosynthesis was a smooth function of chlorophyll concentration and the relation between the two was nearly linear. Finally, it was discovered that photosynthesis reached its maximum rate at about the same light intensity, no matter what the chlorophyll content.

The Chloroplasts

Photosynthesis, so far as we know, does not take place indiscriminately in the assimilating cells, but centres round certain bodies lying in the cytoplasm—the chloroplasts. It is on these plastid bodies that the chlorophyll pigments are fixed. The number of chloroplasts in cells varies very considerably and they also exhibit a great diversity in size and shape. Little is known as to how the chlorophyll is contained on the plastids. Treatment of the chloroplasts with alcohol removes the complex of pigments and leaves a colourless body—the stroma. The nature of the latter is also a matter of controversy; it is usually held to be of protein nature, and by some merely as a denser portion of the cytoplasm.

Origin of the Chloroplasts.—It is usually asserted that chloroplasts arise from pre-existing chloroplasts. This is certainly true for the lower plants, *e.g.*, algæ, mosses and liverworts, in which their fate can be traced through the complete life-cycle. In the zygote of *Spirogyra*, however, they seem to lose their identity and make their reappearance at germination. In the higher plants there is a good deal of uncertainty as to their origin.

Chondriosomes or mitochondria, the small granules, threads or globules which are practically always present in the cytoplasm, have been considered by a number of workers to be the originators

of plastids in the higher plants. Unfortunately our present knowledge of chondriosomes is very meagre and the literature highly conflicting.

In 1910 Lewitsky, working on *Pisum* and *Asparagus*, concluded that the chondriosomes become leucoplasts in the root, and chloroplasts in the stem and leaves. It is difficult to assess the value of this work, but further evidence was brought forward on this point by Cavers (1914), Emberger (1920), Friedrichs (1922), and Alvarado (1923). The most extensive work on this subject

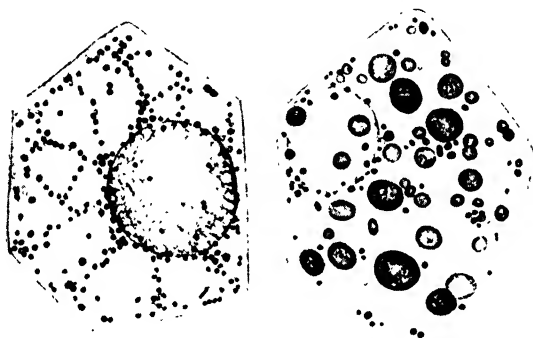


FIG. 38.—Left-hand figure, all from promeristematic region of a germinating seed of maize with small bodies showing as minute granules in the cytoplasm. Right-hand figure, later stage, showing the final stages in the maturing of the chloroplasts. (After Randolph, *Bot. Gaz.*)

has been carried out by Guilliermond (1911–24). According to this investigator, the chondriosomes only arise from pre-existing chondriosomes by division; a view at variance with other workers who consider that they may arise *de novo*. During the development of the plant they perform a variety of functions. Some of them form leucoplasts and chloroplasts, and also elaborate oils and other metabolic products. Mottier (1918, 1921) claimed to have found that in many liverworts and some higher plants, leucoplasts and chloroplasts are derived from rod-shaped chondriosomes, while there is also a second set of bodies which take part

in metabolism. Emberger (1920, 1924) claimed to have found two similar classes in ferns, one of which forms plastids, and the other does not. Kirby (1928) considered that the chloroplasts in the spores of *Osmunda* are formed from chondriosomes or mitochondria.

Randolph (1922) has described the development of plastids from minute primordia in the cytoplasm, and uses the word "proplastids" for these bodies. He found that in the meristematic tissues of young stems and embryonic leaves of *Zea mays*, all the cells contain these proplastids which appear as refractive globules or granules of varying size, the smaller ones showing Brownian movement occasionally being carried about by the streaming of the cytoplasm. Concurrently with the differentiation of the cells, these proplastids gradually enlarge, develop chlorophyll, and are converted into chloroplasts (Fig. 38). Starch makes its appearance within them at a very early stage. Randolph was unable to discover the origin of these bodies, but he concluded that the smallest of these particles were submicroscopic in size.

Zirkle (1926) has studied the chloroplast in the living state, using *Selaginella*, *Elodea*, and a number of other plants. The material was frozen at $-4^{\circ}\text{C}.$, since it was found that freezing does not affect the chloroplasts. Below this temperature the chloroplasts appeared to liquefy and become spindle-shaped and fused into a green meshwork. After freezing, the chloroplasts were examined in monochromatic light of a known wave-length, as every pigment vanishes when examined in light belonging to one of the light bands of its spectrum. The principal investigation was concerned with the chloroplasts of *Elodea*, for, with the exception of *Selaginella*, the general structure of chloroplasts was found to be very similar. According to Zirkle they are hollow, flattened ellipsoids with a central vacuole, which may contain one or more grains of starch. The general granular appearance of the stroma is due to the presence of numerous pores which connect the central vacuole with the cytoplasm surrounding the chloroplast. Around each chloroplast is a more or less permanent sheath of hyaline non-granular cytoplasm. The pigments of the

chloroplasts are intimately mixed and evenly distributed throughout the protein-ground substance; a point confirming Wager (1905), but contradicting Priestley and Irving (1907), who considered that the pigments are distributed round the periphery of the chloroplasts. Zirkle found a certain differentiation of chloroplastids; some contained a little starch and are mainly concerned with photosynthesis, and others included much starch functioned mainly as storage bodies.

Zirkle (1927) has also attempted to trace the origin of chloroplasts. In *Lunularia vulgaris* and *Elodea canadensis* he considered them to originate from primordia indistinguishable from mitochondria. In *Lunularia vulgaris* the plastid development was followed from the apical cell through the mature epidermal cells to cells deep within the thallus. The plastids are considered to originate from these primordia in the apical cell. The primordia are not formed *de novo*, but are formed by division; the division taking place in one plane. In character, the primordia are small, colourless bodies, like prolate spheroids, and are soluble in such fixatives as acetic acid, but are rendered insoluble by treatment with alcohol. A similar state of affairs exists in *Elodea*, but the primordia are smaller and often strung end to end, and may frequently be found grouped round the nucleus. They enlarge, develop chlorophyll, and become plastids. The cells immediately surrounding the apical cell first of all become grass-green, and are no longer soluble in fixatives containing acetic acid.

Andersson (1923) has described a curious situation in a variegated species of the fern *Adiantum cuneatum*. The germination of the spores of this variegated form give rise to two kinds of prothalli, some with normal green chloroplasts and others having small green chloroplasts. The prothalli possessing the small green chloroplasts soon stop developing, and were never observed generally to reach a bigger size than two cells, and rarely more than six. In the meanwhile, the chloroplasts became more and more irregular in shape and the green colour seemed to vanish. Even the prothalli with normal chloroplasts developed more or less pale green patches. They were thus variegated like the sporophyte. These pale patches originate at the growing point,

and the change in colour is a sudden one, any cell containing chloroplasts of only one kind. Both the green and the pale green plastids contain starch, although in the latter the grains are small. All the green cells contain plastids of the same size, and the same can be said of the cells with the pale green plastids; the two kinds were never found to be mixed. This suggests that the cell in some way controls the character of its contained chloroplasts. There is other evidence to the same effect. As regards the mitochondria, it is known that they are separated according as to where they happen to lie when the cell wall is formed in cell division, and the long mitochondrial threads may even be cut across by the cell wall. The change affecting the size of the chloroplasts is sudden, but it is possible that the change affecting colour is primary, and that affecting size secondary.

The nature of the segregation visible in the tissues of the gametophyte constitutes a special problem. The green and white areas in the prothalli presumably differ in their genetic capacities, inasmuch as the prothallus is a haploid structure and genetic segregation occurs after meiosis in which the character of the spores is determined.

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CHAPTER IV

NITROGEN METABOLISM

Source of Nitrogen for the Plant. The Proteins—Iso-electric Point of Proteins—Hydrolytic Products of the Proteins—Constitution of the Proteins—Classification of the Proteins—Protein Synthesis in the Plant—Function of Urea in the Plant—Nitrogen Metabolism of the Leguminosæ—Protein Degradation.

OUR present knowledge of the nitrogen metabolism of plants is still in a very nebulous condition. This is not surprising when it is considered that the actual centre of the synthesis of protein is still a matter of controversy and that the path of translocation of nitrogenous compounds has not been definitely settled. The brilliant investigations of Mason and Maskell (see Chapter II.) have removed the difficulties that once surrounded carbohydrate translocation, but up to the present their work on nitrogen carriage has not yielded any very clear cut or definite results.

The plant physiologist has always been hampered in two respects with regard to plant metabolism, firstly, he has had to investigate a number of complex chemical reactions taking place in a single cell, and secondly, the paths along which substances are carried in the plant have never, till recently, been systematically investigated. It is not too much to say that at present our weapons are insufficiently forged to undertake an attack on the problems of nitrogen metabolism in plants. The whole subject is beset with difficulties; not the least of which is the fact that Abderhalden and his co-workers have recently cast doubts on Hofmeister and Fischer's original conception of the constitution of the proteins.

Ordinary green plants, with the exception of the Leguminosæ, obtain their nitrogen from the soil. Persistent claims have been

made in recent years that wheat is able to assimilate free nitrogen from the air, but these experiments still remain to be substantiated. Nitrates and ammonium salts are considered to be the chief sources of nitrogenous food for green plants. Of the two, nitrates are probably the more efficient. Many fungi, both parasites and saprophytes, have to be supplied with organic nitrogen, although some can utilise mineral nitrogen salts, *e.g.*, *Pyronema confluens* and *Pyronema domesticum* (see Claussen, 1912, and Tandy, 1927). Returning to the higher plants, the study of their nitrogen nutrition is complicated by the difficulty of growing under them sterile conditions in which bacterial decompositions are averted. All normal soils contain active nitrifying organisms which can transform ammonia and ammonium salts into nitrates, and great care has to be exercised in the interpretation of comparative experiments on the value of ammonia or nitrates as a source of nitrogen.

According to Stewart, Thomas and Horner (1925), pineapple plants are able to assimilate all their nitrogen from ammonium salts, although the best growth is made in nitrate solutions. In the early stages of growth the nitrate culture solutions became more alkaline in reaction, but later more acid than the original cultures. The culture solutions containing ammonium salts always showed an acid reaction. A curious fact was discovered in this connection: that plants grown in culture solutions with ammonium salts as a source of nitrogen always contained less calcium than the corresponding nitrate series. It would thus appear that although the pineapple can assimilate ammonium salts it prefers nitrates as a source of nitrogen. Smirnov (1923) grew etiolated barley seedlings in culture solutions containing ammonium chloride, and found an increase of amino-nitrogen, indicating assimilation of ammonia. Calcium increased the assimilation in the preliminary and decreased it in the later stages of the process. The nature of the ammonium salts also has an important influence. Söderbaum (1917) found that ammonium phosphate was more efficient than ammonium sulphate, while ammonium chloride was definitely toxic to some plants. Fred (1924) has found that when barley was grown in sterile sand

culture in which the nitrogen was supplied as ammonium sulphate, the percentage of nitrogen in the crop was very much less than when nitrifying organisms were present. The consensus of evidence, therefore, seems to point to nitrates being the more efficient nitrogenous food for the higher green plants.

It is curious to reflect on the fact that nitrates should be more suitable for the nitrogen requirements of green plants than ammonium salts, since the proteins may be considered as being derivatives of ammonia.

The Proteins

The most important nitrogenous compounds found in living things are the proteins, which form an integral part of protoplasm. It will be necessary here to summarise briefly the more salient chemical and physical properties of these substances for a better understanding of the ensuing discussion on their synthesis by the plant.

The proteins are found in the living portions of all plants. They are present in the dissolved condition in the cell sap, in the semi-dissolved condition in the protoplasm and the solid state in the storage organs, *e.g.*, seeds, roots, bulbs, tubers, etc. In many cells the undissolved protein occurs in well-defined crystals of different shapes, and also in the amorphous condition. The proteins in seeds are the only ones that have been thoroughly investigated, as they occur in these organs in comparatively large amounts and can be more easily isolated. There is at present little evidence available about the nature of the proteins present in metabolically active cells. A step, however, has now been made in this direction by Chibnall and others (see below).

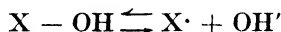
All proteins contain the elements carbon, hydrogen, oxygen, nitrogen, sulphur and sometimes phosphorus and iron are also present. The average composition shows the following values :—

| | | | | | |
|---|---|------|----|------|-----------|
| C | = | 50.6 | to | 55.0 | per cent. |
| H | = | 6.5 | to | 7.3 | „ „ |
| O | = | 21.5 | to | 23.5 | „ „ |
| N | = | 15.0 | to | 19.3 | „ „ |
| S | = | 0.3 | to | 2.2 | „ „ |
| P | = | 0.0 | to | 0.9 | „ „ |

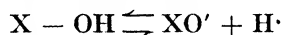
They are colloids, and can be considerably purified by dialysis. It has been found that complete purification by this method is impossible, and it has so far proved impossible to free any protein from traces of inorganic salts. On keeping, they undergo a change known as coagulation. Coagulation can also be brought about by the addition of alcohol, heat and enzymes. Dissolved salts and the *pH* of the medium are also important factors in this connection. A low *pH* is most favourable for coagulation, and a high one may prevent it. They are all *laevo-rotatory*, turning polarised light to the left.

Proteins suffer physical precipitation on the addition of salts to their solutions. No chemical change is produced, and they still retain all their original properties and solubilities. The only proteins not thrown down in this way are the peptones (see below). They give certain characteristic colour reactions with different reagents due to the presence of particular groupings in their molecule, and give precipitates with a number of alkaloidal reagents, such as tannic, phosphotungstic and trichloroacetic acid. They also give insoluble precipitates with the salts of the heavy metals.

The Iso-electric Point of Proteins.—The proteins behave both as acids and bases; in other words, they are amphoteric electrolytes. These amphoteric electrolytes or ampholytes play the part of bases in the presence of acids :—



and with bases they function as acids :—



a phenomenon termed electrolytic tautomerism. At a particular hydrogen-ion concentration the number of protein ions and kations will be exactly equal, this concentration of hydrogen ions is termed the iso-electric point, and the protein will move neither to the anode nor to the cathode, and its solubility at such a point will be at a minimum. The iso-electric point or zone may fall in either an acid or alkaline medium depending on the relative number of acid or basic groups in the protein molecule. If the acid groups be numerous, it will fall in an acid medium, if the

basic groups be numerous it will fall in an alkaline medium. Should both be weak, there will be a wide zone of reaction throughout which the protein will be very slightly combined with either acid or base, and as a first approximation may be regarded as being free from such combination. Pearsall and Ewing (1924A, 1924B) have measured the iso-electric points of a number of plant proteins and have found the following values :—

| | |
|-----------------------------|-----------------|
| Edestin | pH = 5.3 to 5.6 |
| Legumin | „ = 4.4 „ 4.6 |
| Globulin (yeast) | „ = 4.6 |
| Globulin (carrot) | „ = 4.1 to 4.4 |
| Globulin (tomato) | „ = 4.0 |
| Albumin (yeast) | „ = 4.6 |

Chibnall and Grover (1926) have investigated the proteins in the cytoplasm of leaf cells. It was found that the protein in the cytoplasm could be separated into two fractions : (i.) combined proteins, which were in some kind of loose combination with substances soluble in alcohol ; and (ii.) soluble proteins which were uncombined and passed into solution when the cytoplasmic gel was ground with water. These soluble proteins belonged to the class *glutelins* and possessed very similar chemical properties. They had an iso-electric range from pH 4.0 to 5.0 in which their solubility was at a minimum. In all cases the leaf cell sap had a higher pH than the range given above, indicating that the proteins were in the form of anions in the cell.

The Hydrolytic Products of the Proteins

On hydrolysis with acids and enzymes and by fusion with caustic alkalis, the protein molecule is disrupted and gives rise to a number of amino-acids. These amino-acids are bodies in which one or more of the hydrogen atoms (other than carboxyl hydrogen) are replaced by the amino-group — NH₂. The simplest of these amino-acids is glycine or amino-acetic acid, CH₂ . NH₂ . COOH. All the amino-acids, up to the present, that have been isolated from protein hydrolysis belong to the class known as α -amino-acids, *i.e.*, the amino-grouping is attached to the carbon atom immediately adjacent to the carboxyl group.

To preserve the continuity of the argument and also for convenience of reference, the principal amino-acids isolated from the proteins are appended in the following list :—

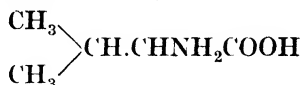
Glycine (α -amino-acetic acid) $\text{CH}_2\text{NH}_2\text{COOH}$

Alanine (α -amino-propionic acid) $\text{CH}_3\text{CHNH}_2\text{COOH}$

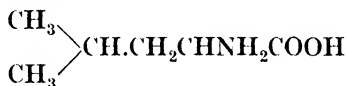
Amino-butric acid $\text{CH}_3\text{CH}_2\text{CHNH}_2\text{COOH}$

Amino-caproic acid $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CHNH}_2\text{COOH}$

Valine (α -amino-isovaleric acid)



Leucine (α -amino-isocaproic acid)



Isoleucine (α -amino- β -methyl- β -ethyl propionic acid)



Serine (α -amino- β -hydroxypropionic acid)



Aspartic (α -amino-succinic acid) $\text{COOH} \cdot \text{CH}_2\text{CHNH}_2\text{COOH}$

Glutamic (α -amino-glutaric acid) $\text{COOH} \cdot \text{CH}_2\text{CH}_2\text{CHNH}_2\text{COOH}$

Ornithine (α - δ -diamino-valeric acid)



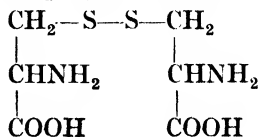
Arginine (δ -guanidine- α -amino-valeric acid)



Lysine (α - ϵ -diamino-caproic acid)



Cystine (di[β -thio- α -amino-propionic] acid)



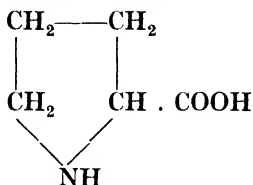
Phenyl alanine (β -phenyl- α -amino-propionic acid)



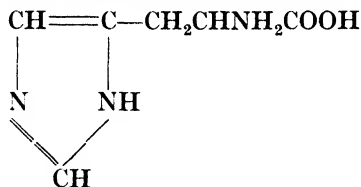
Tyrosine (β -*p*-hydroxyphenyl- α -amino-propionic acid)



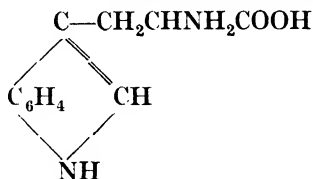
Proline (α -pyrrolidine carboxylic acid)



Histidine (β -imidazol- α -amino-propionic acid)



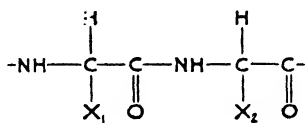
Tryptophane (β -indole- α -amino-propionic acid)



The Constitution of the Proteins

Although the amino-acids are the cleavage products of proteins and are obtained by their hydrolysis, yet the proteins themselves give very little free nitrogen when treated with nitrous acid; a characteristic of the amino-acids, which give a quantitative yield of nitrogen when so treated. Arguing from this and other facts, Emil Fischer put forward the view that the protein molecule is

built up of chains of amino-acids, in which the amino-grouping of one acid is combined with the carboxyl group of another :—

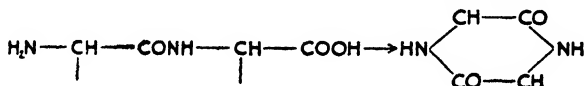


where X_1 and X_2 represent different amino-acid radicles.

Such compounds have been synthesised in the laboratory and are known as peptides. An example of a peptide is glycylglycine, $\text{CH}_2\text{NH}_2\text{CO} \cdot \text{NH} \cdot \text{CH}_2\text{COOH}$. Since this is formed from two amino-acids it is termed a dipeptide. Tripeptides are formed from three amino-acids, and the series can be extended till compounds are reached with many amino-acids in their molecules; these are called polypeptides. Fischer was able to synthesise a polypeptide with eighteen amino-acid radicles in the molecule and Abderhalden has obtained one with nineteen such radicles, and a molecular weight of 1,200.

These synthetic products show a close resemblance to the natural peptones. The majority are soluble in water, and (with the exception of certain *di*- and *tri*-peptides) give the biuret reaction, a blue colour with alkaline copper sulphate solution. They are precipitated by phosphotungstic acid and have a bitter taste like many natural peptones. They are readily hydrolysed by acids, and they can also in many cases be hydrolysed by proteolytic enzymes. The closest resemblance is found to the proteins in those peptides which have a long chain composed of different amino-acid radicles.

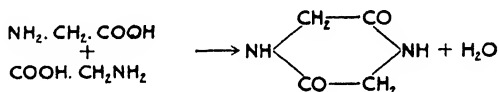
There is no reason to suppose that the amino-acids in the protein molecule are not linked in other ways as well. Fischer himself suggested ring formation of the following type :—



showing the possibility of piperazine group being present in the molecule and ring formations of this type are readily accounted

for by the elimination of water from a complex of two amino-acid molecules.

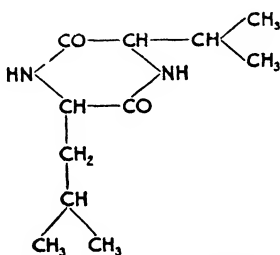
Fischer's views on the constitution of the proteins were unquestioningly accepted for over twenty years and undoubtedly played an enormous part in the furtherance of the study of these complex substances. But recently, Abderhalden has introduced a new conception of the constitution of the protein molecule. For a summary of his views the review by Klarmann (1927) should be consulted. The matter can only be briefly dealt with here, and Abderhalden's original papers are too numerous to be given adequate mention in a text of this nature. Abderhalden pointed out the well-known fact that the cleavage products of the proteins by chemical means or peptic and tryptic enzymes has often yielded compounds with cyclic and not straight polypeptide chains, *e.g.*, 2 : 5-dioxopiperazines have been found after protein degradation. These 2 : 5-dioxopiperazines are composed of two amino-acids and their simplest representative is glycine anhydride, which is formed if an aqueous solution of glycine is left standing for some time :—



The two amino-acids concerned may be the same or different, and they may also differ in their spacial configuration. Thus a great variety of these cyclic products are possible. Abderhalden regarded the protein molecule as a complex polymeride of these cyclic compounds.

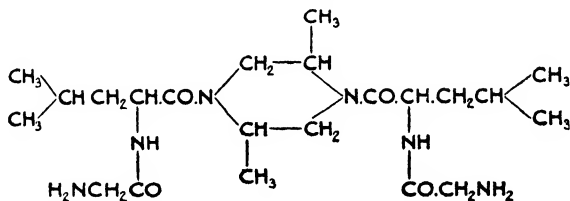
Two views are possible in connection with these substances : (a) they exist as such in the protein molecule ; and (b) their formation is secondary and occurs after the breakdown of the molecule. By partial hydrolysis of the protein, concentration of the product *in vacuo* and subsequent extraction with ethyl acetate, Abderhalden and his workers have been able to isolate a number of new diketopiperazine derivatives. Extreme care must be exercised in these experiments, so that diketopiperazine formation by secondary condensation is excluded. From the

chemical standpoint these diketopiperazines are rather resistant towards acids. Abderhalden isolated *d*-valyl-*l*-leucine anhydride by the hydrolysis of casein with 5 or 10 per cent. sulphuric acid and extraction of the dry product :—



d-VALYL-*L*-LEUCINE ANHYDRIDE

Abderhalden contended it improbable that this procedure should lead to secondary formation of the anhydride. Further evidence in favour of his views on protein constitution comes from their oxidation products with potassium permanganate and reduction with sodium and alcohol. Both these treatments give oxamide, though in the reduction process the yield is very small. He has also been able to synthesise these substances, and has obtained, for example, diglycyl-leucyl-2 : 5-dimethylpiperazine :—



Diglycyl-leucyl-2 : 5-dimethylpiperazine

But neither this nor any of the synthetic products obtained by Abderhalden were attacked by proteolytic enzymes.

Osborne and Vickery (1928) have strongly criticised Abderhalden's views on protein constitution, and although they admitted that the mass of evidence brought forward by him is impressive, yet they very rightly recalled the fact that without exception all the synthetic products so far obtained have been quite resistant

to resolution by enzymes. Fischer's products, on the other hand, were resolved in a number of cases by trypsin, and this fact alone is a very strong argument in favour of the retention of the old polypeptide hypothesis. It does appear from the present state of the subject that a great deal more evidence must be forthcoming before Abderhalden's views can be unreservedly accepted.

The Classification of the Proteins

At present, there is no very satisfactory classification of the proteins. The best, perhaps, is the scheme of the American Committee on Protein Nomenclature which is given below :—

I. Simple Proteins.

- (a) Albumins.
- (b) Globulins.
- (c) Glutelins.
- (d) Prolamines.
- (e) Albuminoids.
- (f) Histones.
- (g) Protamines.

II. Conjugated Proteins.

- (a) Nucleoproteins.
- (b) Glycoproteins.
- (c) Phosphoproteins.
- (d) Hæmoglobins.
- (e) Lecithoproteins.

III. Derived Proteins.

(i.) Primary protein derivatives.

- (a) Proteans.
- (b) Metaproteins.
- (c) Coagulated proteins.

(ii.) Secondary protein derivatives.

- (a) Proteoses.
- (b) Peptones.
- (c) Peptides.

Of these proteins, the glutelins and prolamines are only represented in the vegetable kingdom and the albuminoids and protamines are characteristic products of animals.

Only the properties of the larger groups will be considered here as far as these bear on the discussion of protein synthesis in the plant. For a full description of the properties of the vegetable proteins, the reader should consult the monograph by Osborne (1924).

Albumins.—These are not very abundant in plants. They are readily soluble in water and coagulated by heat. They are not precipitated by sodium chloride or magnesium chloride, but are precipitated by a saturated solution of ammonium chloride. The best characterised vegetable albumins are leucosine in the grain of cereals, legumelin in soya-bean, cowpea and lentil, phaselin in the kidney bean and ricin in the castor bean.

Globulins.—These are proteins insoluble in water, but soluble in weak saline solutions. They are readily coagulated by heat, and precipitated from solution by magnesium sulphate and half-saturated ammonium sulphate.

Glutelins.—This group includes those proteins which are not dissolved by neutral aqueous solutions, saline solutions or alcohol. They are soluble in weak alkali or acid. Glutelin occurs in wheat and oryzenin in rice.

Prolamines.—This is a group of proteins characterised by their solubility in 70 to 90 per cent. alcohol. On hydrolysis they yield large amounts of glutamic acid, proline and ammonia, small amounts of arginine, histidine and little or no lysine.

Certain derivatives of the proteins can also be considered here. The metaproteins, for example, obtained by the hydrolysis of protein by trypsin or pepsin. The metaprotein obtained is termed acid or alkali albumin depending on whether pepsin or trypsin is used for the hydrolysis, since the former works in alkaline, and the latter in acid media.

In general terms, it can be said that similar proteins are found only in seeds of the same natural family. This has an important influence on the subsequent development of the embryo, which in the first stages of germination obtains its nitrogenous food by the

hydrolysis of the reserve proteins present in it. This protein is one of the final products of the metabolism of the plant, and, in the primary stages of development, the embryo is supplied with a definite food, which for each member of the same species is the same. Each member of a species thus begins its individual life under similar chemical conditions which are different for those of any other species. It would thus appear that when the green plant has reached the stage of development when it obtains its food from the soil and air its chemical processes have already been established along certain definite lines which it must follow for the rest of its life-cycle.

Protein Synthesis in the Plant

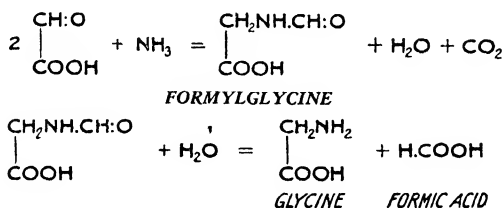
It is generally assumed that in the higher green plants the chief seat of protein synthesis is the leaves. In 1890 Sapoznikoff established the fact that there was an increase of protein with increase of carbohydrate in the leaf in the presence of light. It has also been discovered that nitrates accumulate in leaves in the dark and disappear in the light. In variegated leaves it is only from the green portions that nitrates disappear. It was therefore concluded that leaves were the chief centre of protein synthesis and that the process could only take place in the light.

Light, however, is not apparently a necessary factor. In 1901 Zalesski found that protein synthesis could occur in leaves in the dark in the presence of nitrates and carbohydrates. The necessary factor for the synthesis appeared to be carbohydrate and not light. The function of the light was to give to the leaves the power of photosynthesis, and thus only indirectly helped in the synthesis of proteins. Robinson (1929) has criticised this work on the grounds of technique. The protein was estimated by the method of Stutzen, but amino-acids and carbohydrates can combine at low temperatures to give complexes precipitable with copper sulphate, so that the increase registered may have been due to this cause and not to true protein nitrogen. But the recent investigations of Muenscher (1923) have more firmly established the fact that protein synthesis can take place in the dark and that light is not a necessary factor. He grew *Chlorella*

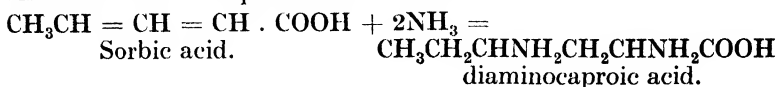
in nutrient solutions containing nitrogen, either as calcium nitrate or ammonium sulphate in diffuse light, and also in total darkness for 105 to 235 days. Quantitative determinations were made of the volume, dry-weight and total nitrogen, and these showed strong evidence that the alga could synthesise protein in the absence of light when nitrogen was supplied in the form of inorganic salts.

It has already been stated that nitrates form the starting-point of protein synthesis. But nitrates, chemically speaking, are simple bodies, whereas the proteins are complex bodies of high molecular weight. Adair (1924) has calculated that albumins have a molecular weight of 68,000 and globulins a still higher value, 150,000. It is obvious, therefore, that the process must take place in stages. Treub put forward the suggestion that hydrocyanic acid was a possible intermediate stage in the synthesis. The suggestion carries little weight, as free hydrocyanic acid has not been found in plants.

An alternative view is that amino-acids are first formed, and these are later condensed to give proteins. The synthesis of amino-acids has been brought about in the laboratory by the direct action of ammonia on aliphatic and aromatic acids. Erlenmeyer and Kunlin were able to synthesise formylglycine by the action of ammonia on glyoxylic acid :—



The formylglycine is readily hydrolysed to glycine and formic acid. Again, Fischer and Schlotterbeck synthesised diamino-caproic acid by the interaction between ammonia and sorbic acid, an unsaturated acid present in the berries of the mountain ash :—



It should be noted that these products are α -amino-acids. Nevertheless, it is a far cry from such laboratory reactions to the synthesis of these acids by plants, and it is in the highest degree unsafe to draw analogies between test-tube reactions and the chemical reactions of the living cell.

Although light does not appear to be an essential factor for protein synthesis, the presence of potassium seems to be necessary. Beet, for example, forms less protein in the absence of potassium. Although protein nitrogen is less in such circumstances, it has been shown by Burrell (1926) that there is an accumulation of amino-acids. The cell appears to lose its power of converting the amino-acids into protein in the absence of potassium. Calcium is also an important factor, and in the absence of this element there is a considerable accumulation of nitrate in the cell. (See section on Calcium in Chapter VI.)

In connection with the question of nitrates and their relation to protein synthesis, it must be remembered that the nitrates are, relatively speaking, stable bodies, and comparatively inert. They must therefore be brought into a more reactive condition. The probabilities are that the first stage of the synthesis of protein in the living cell is the conversion of nitrate into nitrite. This was first demonstrated in 1890 by Laurent and later confirmed by Hankinson and Irving (1908) for the leaves of *Sagittaria*. Anderson (1924) found nitrate to be present in the shoots of about forty different species of plants as well as in the seeds of *Brassica nigra*, *Cannabis sativa* and *Lepidium sativum*. The amount of nitrate in the plants appeared to vary with the season. Thus in *Mercurialis perennis*, positive reactions were obtained in October and negative in June, whereas material tested for nitrite in October gave negative results and positive results were obtained in June, i.e., the reverse of the nitrate. The presence of nitrate in the plant, according to Anderson, appears to depend to a considerable extent on the richness of the soil. Thus she found that plants growing in highly manured soil gave a strong positive reaction, possibly because they had absorbed more nitrate than they could elaborate in a given time.

Anderson showed that there is some kind of nitrate-reducing

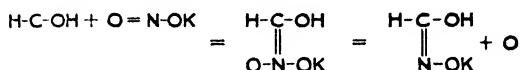
mechanism in the plant by the following experiment: 10 drops of a 4 per cent. solution of sodium nitrate was added to 10 c.c. of expressed sap, and the liquid was then equally divided between three test-tubes. To one of the tubes was added 2 drops of acetaldehyde (10 per cent.), and all three tubes were then placed in a water bath at 45° C. The tube without the aldehyde was removed at the end of ten minutes, and the other two at the end of twenty minutes. After cooling, the extract was half saturated with ammonium sulphate, filtered to remove chlorophyll and tested for nitrite with Greiss-Ilosvay reagent. The tube with acetaldehyde gave a positive reaction. The actual substance responsible does not appear to be an enzyme, but is of the nature of the oxidisable substance "atite" discovered by Haas and Hill (1923) in milk, and which reduces nitrate to nitrite. Anderson considered it doubtful if this substance plays any part in protein synthesis, since it is only under artificial conditions of high temperature (45° C.) and in the presence of aldehyde that the reduction takes place. It is unfortunate that this work was never carried any further and the influence of iron investigated on the reducing mechanism, for Eckerson (1924) has shown that the juice extracted from various parts of the tomato possesses the power of reducing nitrates to nitrites and finally gives ammonia. This process can occur in either light or darkness and boiled extracts will also bring about the change, provided that the pH of the medium is kept at 7.6. It was discovered that the important factor in this reducing process was the presence of iron.

It has been known for a considerable time that nitrates can be reduced to nitrites photochemically, *i.e.*, under the influence of light, but this change will not occur in the dark. The situation is therefore a curious one: protein synthesis can take place in the dark provided that carbohydrates are present, yet nitrites are produced from nitrates photochemically. Eckerson's investigation, however, goes a long way to show that the plant possesses some mechanism whereby nitrates can be reduced to nitrite and even ammonia in the absence of light, and light therefore does not enter as a factor into protein synthesis.

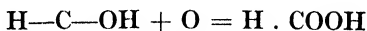
Nevertheless, Baly, Heilbron and Hudson (1922), and Baly,

Heilbron and Stern (1923) assumed that protein synthesis is a photochemical process, and claimed that the first stage in the reaction is the production of active aldehyde from carbon dioxide and water, and nitrite from nitrate under the influence of light.

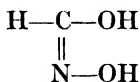
The nitrite and active aldehyde then combine to give formhydroxamic acid as the potassium salt, one atom of oxygen being split off in the course of the reaction :—



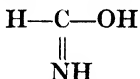
The oxygen liberated is said to oxidise some of the active aldehyde to formic acid :—



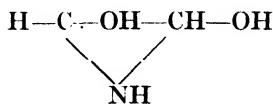
Under the experimental conditions used by these investigators, the formhydroxamic acid was alleged to be completely hydrolysed to the free acid :—



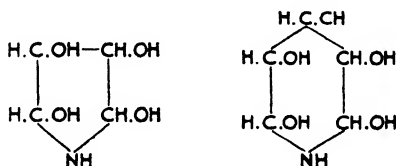
This compound was considered to lose oxygen to give :—



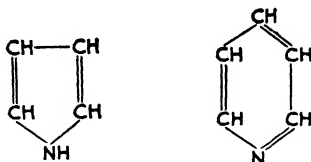
Such a substance could condense with more active aldehyde to yield first :—



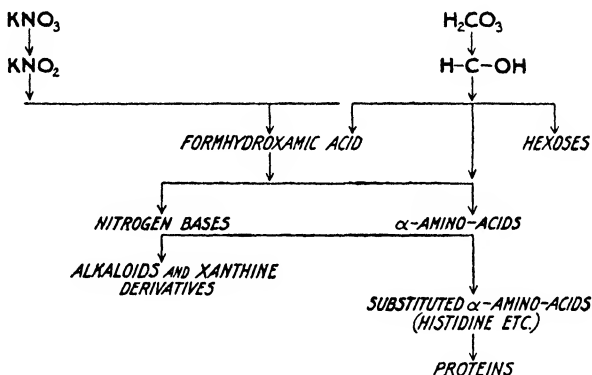
and this by intramolecular change would give glycine, $\text{CH}_2\text{NH}_2\text{COOH}$. It could also condense with either three or four molecules of active aldehyde to give cyclic derivatives :—



these, by loss of oxygen and water, would give pyrrole and pyridine derivatives :—



Baly, Heilbron and Hudson have summarised their results in the following scheme :—



These reactions are all based upon *in vitro* experiments. Baly and his co-workers presupposed light to be an essential factor. It has already been seen that light is not a necessary factor, and it is the presence or absence of carbohydrates that is necessary. In any case, this work has been submitted to such drastic criticism, both in this country and also in America, that little reliance can be placed upon it.

Schimper showed, many years ago, that the reduction of nitrates to nitrites in leaves was in some way connected with the presence of iron compounds and not with light, since chlorotic leaves with no iron failed to reduce nitrates. It has since been shown by Baudisch (1921, 1923) that the cholera bacillus in peptone culture has the power of accumulating iron within it in large amounts, and it also possesses the power of reducing nitrates

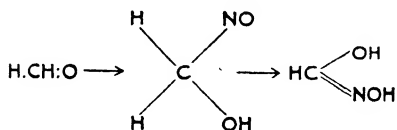
to nitrites to an altogether remarkable extent. Moreover, it was discovered that this reduction was directly correlated with the iron content and oxygen respiration. Baudisch has also demonstrated that one of the oxygen atoms in the nitrate molecule is differently combined to the others and very much more readily split off, either under the influence of light or iron to give nitrite, the reactions being fundamentally the same and the iron functioning as a catalyst.

Baudisch found that aqueous solutions of potassium nitrite containing easily oxidisable substances such as ethyl alcohol, aldehydes, sugars, starches, etc., suffer a comparatively rapid reduction under the influence of diffused daylight, the nitrite being converted into potassium nitrosyl and oxygen :—

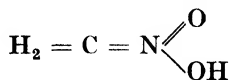


Complex iron salts were also found to bring about the reaction. Thus, in the presence of a trace of iron, a faintly alkaline solution of glucose and potassium nitrite gave potassium nitrosyl and ammonia. But nitrates could not be reduced in this manner. Apparently the reduction of nitrate in biological reactions needs the presence of oxygen, and it has long been known that ferrous salts in the presence of oxygen instantly reduce nitrates to nitrites.

Nitrosyl itself readily reacts with formaldehyde to give formhydroxamic acid, with the intermediate production of nitroso-methyl alcohol. The latter, by intramolecular rearrangement, gives the formhydroxamic acid :—

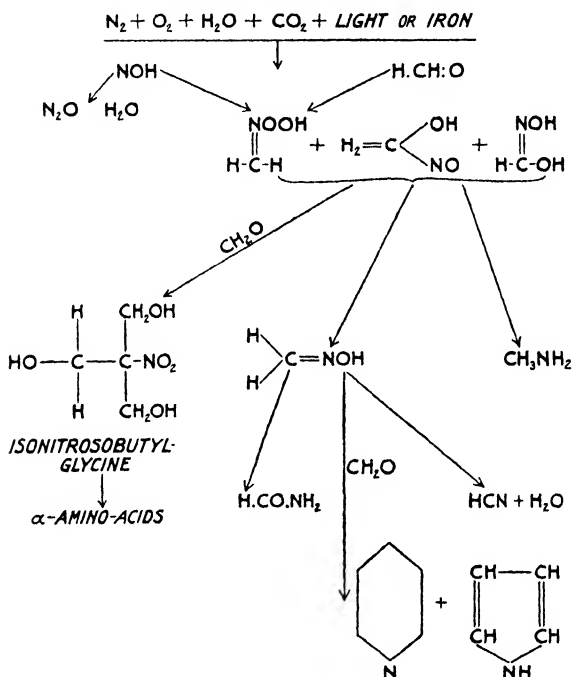


Formhydroxamic acid is quite stable in the dark, but in the presence of light changes over partly to aci-nitroso-methane :—



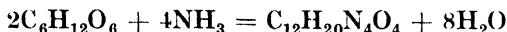
Longer exposure to light yields methylamine, CH_3NH_2 .

Baudisch, by means of colour reactions, discovered that under the influence of light the formhydroxamic acid gives formaldoxime, and a second portion gives hydrocyanic acid and water. The presence of the oxime was found in peptone cultures of the cholera bacillus. The anhydrous product rapidly polymerises to the tri-molecular form. In weakly alkaline solution and in light it undergoes intramolecular change with the formation of an intermediate labile compound which is capable of linking itself with more formaldehyde with the formation of complex nitrogen cyclic derivatives. Summarising these results we have :—

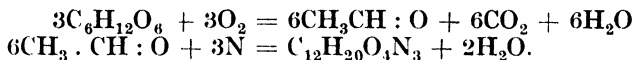


It is possible, as Baudisch suggested, that under the influence of nitrosyl, amino-nitrogen is introduced into the higher alcohols, sugars, etc., to give amino-acids, since it is so easily able to enter into different combinations, and that the amino-acids are subsequently condensed to give the proteins.

In the yeasts, Effront (1927) claimed that the synthesis of proteins depends on carbohydrate supply, and takes one course under aerobic conditions and a different course when oxygen is excluded. Under anaerobic conditions the following reaction takes place :—



According to quantitative determinations there was a transformation of glucose through pyruvic aldehyde (methylglyoxal) to a polypeptide. The pyruvic acid first gave alanine, and this was then condensed to the polypeptide. In the presence of oxygen, acetaldehyde, carbon dioxide and water were first produced, and these combined with active nitrogen to give a polypeptide :—



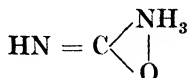
The Function of Urea in the Plant

It is a well-known fact that urea plays an important part in the metabolism of animals. It is chiefly in the form of this compound that the nitrogenous waste products of protein metabolism of animals is removed in the urine. The presence and function of urea in plants, however, has always been a matter of controversy. But the enzyme, urease, which eventually decomposes urea into ammonia and carbon dioxide has a wide distribution in the vegetable kingdom, especially among the Leguminosæ, and if this enzyme be present, it would appear improbable that urea is not present as well.

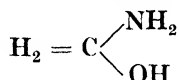
Urea is undoubtedly present in the fungi. Iwanoff (1923) has made a number of quantitative investigations on this question and shown that urea is present in *Lycoperdon saccatum*, *L. piriforme*, *L. gemmatum*, *Bovista nigrescens*, and *Psalliota campestris*. For example, as much as 11 to 16 per cent. of the dry-weight of *B. nigrescens* was found to be composed of urea. The urea was shown to be absent in the early stages of development and increased to a maximum with age. In *Lycoperdon*, Iwanoff showed that the urea was formed from available ammonia, and considered that its function in the fungi was to act as a nitrogen reserve. Fosse

(1916, 1920) claimed that urea was also present in the higher green plants, and claimed to have demonstrated its presence by the delicate xanthohydrol reaction.

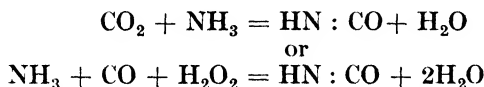
Werner (1922) who has succeeded in showing that urea possesses the cyclic constitution :—



which can in certain circumstances give the open ring structure :—

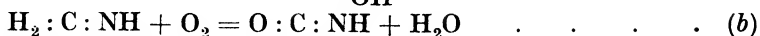
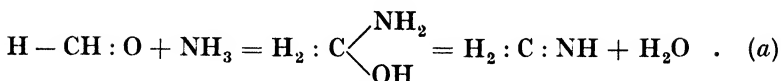


considered that in the germinating seedling when the assimilation apparatus has become fully established cyanic acid is built up from carbon dioxide and ammonia according to the following equation :—



and that the subsequent condensation of this cyanic acid with nascent carbohydrates is the first step in the synthesis of proteins. It has been known for a long time that plants can assimilate urea from culture solutions, but not the substituted ureas, such as ethylurea. It is possible that plants cannot utilise the substituted ureas on account of the fact that they do not possess any enzyme in their cells capable of hydrolysing these bodies to ethylamine and cyanic acid, whilst the superior action of urea over ammonia in this respect is due to its power of immediately supplying cyanic acid.

The production of cyanic acid from ammonia also requires consideration. Werner assumed that the production of cyanic acid from ammonia is slower in the presence of carbohydrates at the ordinary temperature than the decomposition of urea to cyanic acid by urease. He further assumed that formaldehyde is the first product of photosynthesis, and considered that the reaction between the former and ammonia may proceed as follows :—



Reaction (b) was considered to be brought about under the influence of oxidising enzymes, such as oxidase. The cyanic acid produced was supposed to be converted into protein by combination with nascent carbohydrate.

The Nitrogen Metabolism of the Leguminosæ

The position of the Leguminosæ is peculiar with regard to their nitrogen metabolism. It has been proved beyond all doubt that



FIG. 39.—The Life Cycle of *Bacillus Radicicola* (After Thornton and Gangulee, *Proc. Roy. Soc. Lond.*)

these plants can assimilate the free nitrogen of the atmosphere. This is due to the presence of the bacterium, *Bacillus radiculicola* in the nodules on the roots of these plants. This organism has been shown by Bewley and Hutchinson (1920) to pass through a very definite life-cycle. They showed that the cycle started with non-motile cocci which swelled and developed flagella. The next stage consisted in the loss of the flagella and the bacteria again became non-motile and vacuolated. In the final stage the bacteria showed a banded appearance and then passed into cocci once more or developed flagella (Fig. 39). This work has been

confirmed by Thornton and Gangulee (1926), who showed that the same series of transformations occurred in the soil as well as in artificial culture. In any sample examined, all these cell types occur, but their proportions vary at different times, and the changing percentages in the first twenty-seven hours is shown in Fig. 40. Within this period the population passed through two

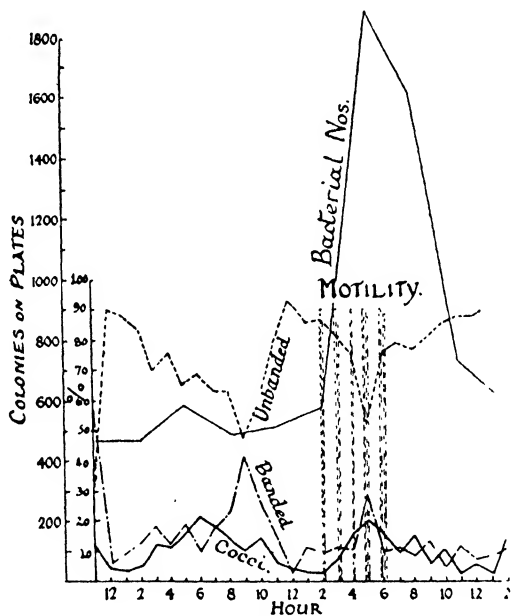


FIG. 40.—The total number of organisms and percentages of cocci, banded and unbanded rods, at two-hourly intervals in culture. (After Thornton and Gangulee, *Proc. Roy. Soc. Lond.*)

complete cycles of change, each commencing with an increase in the proportion of cocci and banded rods. In both cycles, the maximum percentage of cocci was reached as soon as, or before, that of the banded rods from which they arose. The presence of di-acid calcium phosphate (0.1 per cent.) in milk hastened the predominance of the cocci and increased their percentage. The presence of calcium also affected the migration of the bacteria through the soil and helped in their wider distribution. The

effect was chiefly shown towards the tips of the roots rather than near the seed. The precise influence of the phosphate was not discovered.

The presence of minute amounts of boron also have an important effect. In the absence of boron the bacterial nodules do develop extensively and the bacteria tend to assume a parasitic habit (see Chapter VI.).

The bacteria enter the plant *viâ* the root-hair near the tip and multiply with considerable rapidity, and this is followed by much softening of the wall of the hair. They are strictly confined to the inner cells of the cortex. Strands grow down from the main vascular system and connection is made with the circulating system of the plant. During entry the bacteria pass through the cycle described above. The non-motile cocci enter the root-hair as swimmers. Once in the interior of the hair, they become rods, and finally become branched banded rods in the nodule.

The chemistry of nitrogen fixation is still quite unknown. The plant is supposed to be supplied with soluble protein, but the stages whereby the molecular nitrogen of the atmosphere is transformed into complex protein have still to be solved.

Protein Degradation

Protein degradation embraces two problems. The first is the fate of the elaborated protein in the leaf, and the second that of the reserve protein of seeds and storage organs. It will be simpler to discuss these problems separately.

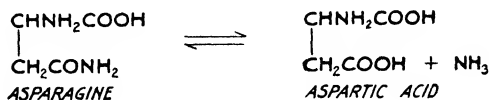
Protein synthesised in the leaf must be translocated away to the growing points, storage organs and developing seeds. The plant, unlike the animal, does not actively excrete waste nitrogen as urea in the urine, and the ultimate fate of the protein elaborated in the leaf takes a different course to that exhibited in the animal. Thomas (1927) has made an elaborate study of the nitrogenous metabolism of apple trees. The distribution of nitrogen in the water-soluble fractions of both leaves and shoots were investigated at intervals throughout the growing season, and in a subsequent season a comparison was made between fertilised and unfertilised

trees, *i.e.*, those receiving a heavy dressing of sodium nitrate and others not treated in this way. An examination of nitrogen distribution appeared to indicate that a single protein was present in the leaf; though there could be no absolute certainty about the matter. The changes throughout the year are interesting. When growth is rapid the nitrogen tends to migrate from the leaves to the shoots, where it is stored in the phloem. During bud formation, the reserve protein is transported to the actively growing centres as amino-acids. In the autumn there is migration of nitrogen from the leaves to branches, and it is stored mainly in the first or second year shoots. The removal of nitrogen from the leaves to the shoots in the autumn has always been a long-standing controversy and is seemingly now settled by this work.

Chibnall (1922, 1923), from determinations based on wet weight measurements, found a well-marked diurnal variation in the nitrogen content of the leaves of *Phaseolus vulgaris*, and concluded that there was a continuous decomposition of protein by day as well as by night in the leaf with a simultaneous withdrawal of the soluble products of this decomposition to other parts of the plant. The process was masked during daylight, because the amount of synthesis was greater than that of degradation.

It was first shown by Hartig in the middle of the last century that the amide, asparagine, $\text{COOH} \cdot \text{CHNH}_2 \cdot \text{CH}_2\text{CONH}_2$, was produced in considerable amount as a decomposition product of plant proteins. Chibnall put forward the view that there is a continuous production of asparagine from protein in the normal mature leaf, and that in plant metabolism, asparagine plays the rôle of a translocatory substance, and is the chief medium whereby nitrogen, in a form suitable for subsequent resynthesis can be conveyed from one part of the plant to another. According to Pranschnikoff (1924A, 1924B, 1926), asparagine is formed as a temporary nitrogen reserve whenever there occurs, through some derangement of normal protein metabolism, an excess of ammonia which is toxic to the plant. This ammonia is rendered harmless by condensation with aspartic acid to form asparagine. Unfortunately, the reactions whereby protein and asparagine are interconverted are still very far from clear. The degradation of

proteins through proteoses, polypeptides and amino-acids is well known, but there is little evidence regarding the steps between amino-acids and asparagine. It has usually been assumed that the immediate precursor of asparagine is aspartic acid, and that an enzyme, asparaginase, controls the reaction :—



The presence of aspartic acid in the plant has not been demonstrated, although Kiessel (1924) has put forward a claim that he has isolated this amino-acid from the ears of ripening rye. As no asparagine was discovered even in the later stages of ripening, he considered that aspartic acid could not be the forerunner of asparagine, and that de-amidation of the asparagine must take place at a stage earlier than those he investigated. Grover and Chibnall (1927) pointed out that investigators have failed to differentiate between de-amination and de-amidation, and that the evidence of the existence of an enzyme asparaginase is very meagre and uncertain. They have isolated an enzyme, from the rootlets of germinating barley, that is capable of liberating ammonia from asparagine. A preparation was made by grinding up the dried rootlets to a fine powder and making this into a thick cream with water. Such a preparation was found to release ammonia from asparagine at a pH of 7.5. The enzyme itself was isolated by precipitation from an aqueous solution with alcohol.

This enzyme is specific in nature and will only attack *l*-asparagine and not the *d*-form to give aspartic acid. It will also attack *d*-glutamine, which is closely related to asparagine. Other amides, such as acetamide and propionamide were not hydrolysed. The enzyme also attacked glycylglycine with liberation of ammonia. There is thus no question that an enzyme exists in the higher plants capable of effecting de-amidation of asparagine. By adopting the view that the amide group of asparagine is essentially a peptide linkage and that both asparagine and glycylglycine are

hydrolysed by a barley peptidase, our conception of protein metabolism is considerably simplified.

Mothes (1926) has found that protein synthesis is more active in young leaves than in mature ones, and in the latter protein hydrolysis plays a major rôle. Mothes concluded from his results that asparagine production is a secondary process due to oxidation and has nothing to do with translocation. He considered that its function is to hold and bind the ammonia produced in protein hydrolysis, which, if it were allowed to accumulate, would be toxic to the cells.

Woodman and Engeldow (1924) have studied the development of the wheat grain with special reference to the synthesis of proteins in the grain itself. They could find no trace of nitrate in the development of the grain, and have concluded that the reduction of nitrate to ammonia must take place at some stage exterior to the grain and that the nitrogen enters the grain as asparagine or ammonium compounds or both, and is there synthesised to protein.

The seeds and storage organs of plants contain reserve protein, and these have to be brought into a suitable condition for the metabolic activities of the newly developing plant. Etiolated plants and actively growing plant organs are very suitable for a study of protein hydrolysis as the problem is not complicated by protein synthesis. In the animal, proteins taken as food are hydrolysed in the alimentary canal to amino-acids, and are reconstructed to form protein in the various tissues after their passage through the walls of the intestine into the blood stream. The same series of reactions is apparently followed by the developing seedling.

Zlateroff (1916) has found that after twenty days' germination, *Cicer arietinum* showed a lower content of protein and higher content of amino-acids. Stark (1927), working with two varieties of soya-bean, Manchu and Midwest, discovered that two maxima exist for the amino-acid content. The first occurred during the active growth of the hypocotyl and the second during the development of the epicotyl. In etiolated seedlings, marked fluctuations occurred in amino-acid content, and the curve showed a series of

modes. In the early stages of development temperature plays an important part in protein hydrolysis, while in the later stages of development it has no effect.

An acute controversy still exists as to the function of asparagine in the developing seedling. Sure and Tottingham (1916) found in the pea that there was an accumulation of asparagine in the shoots of the developing seedling and that there was a marked increase in amide nitrogen with concurrent decrease in amino-acid nitrogen. In any case asparagine is abundant in developing seedlings. On the older view it was considered that the reserve protein broke down to asparagine, and nitrogen is translocated in this form to the developing parts of the young plant and re-synthesised into protein. This view was supported by Chibnall. Pranschnikoff, however, was in disagreement with such a theory, and considered that the function of the asparagine is to bind toxic ammonia produced in protein hydrolysis. Definite evidence in favour of one or other of these two views is still wanting, and little profit can accrue from its discussion.

In bringing this discussion to a close, the review of the whole of the evidence of protein synthesis and protein degradation in the living plant shows a lamentable lack of knowledge. This is doubtless largely due to the fact that the problem has only been spasmodically investigated by independent workers who have paid little regard to the work of others. The problems that have to be faced are undoubtedly complex, and it is certainly a misfortune for plant physiology that it has been tinkered at in this way.

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CHAPTER V

RESPIRATION

Chemistry of the Production of Organic Acids in Respiration—Carbon Monoxide as a Product of Respiration—Nature of Aerobic Respiration—Factors affecting Respiration—Anæsthetics—Anaerobic Respiration and Fermentation—Oxidation Mechanism of the Cell—Enzymes concerned in Respiration—Oxidation of Fats and Proteins in the Plant.

THE main advances in the study of plant respiration have been extended in a variety of different directions.

Although in normal respiration carbon dioxide and water are the end products of the reaction, it has been known for a number of years that certain succulents, notably the Cactaceæ, Crassulaceæ and Mesembryanthemaceæ, produce organic acids as the final products of respiration. This result may well be due to the massive construction of these plants, making gaseous diffusion a difficult process. The production of organic acids in respiration, however, has been mainly investigated from the biochemical standpoint in the bacteria and fungi.

A curious point has been discovered in this connection, namely, that the bacteria produce monobasic acids, whereas the fungi give polybasic acids of the type of fumaric, citric and oxalic acids. This aspect of the problem has been investigated for the ascomycete, *Aspergillus niger*, and it has been known for a very considerable time that this fungus produces large amounts of oxalic acid as a product of respiration. The work of Wehmer, conducted in the 'nineties, showed that not only was oxalic acid produced from glucose, but also from the salts of organic acids, such as tartaric, citric and malic acids. He found that the production of oxalic acid was independent of the supply of oxygen, but was intimately connected with temperature; the higher the temperature, the greater the production of acid. If the acid were

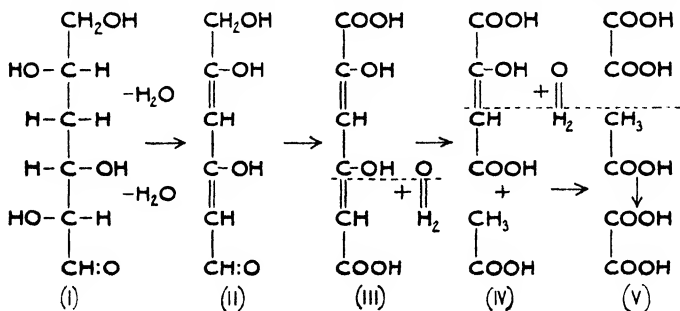
fixed in the form of the calcium or sodium salt by the addition of the carbonates of these metals to the medium, he showed that the fungus did not appreciably attack the salt of the acid, whereas the free acid was eventually oxidised to carbon dioxide and water.

According to Currie (1917) the stages in the decomposition of carbohydrate to oxalic acid by *Aspergillus niger* are as follows :—
Carbohydrate \rightarrow Citric acid \rightarrow Oxalic acid \rightarrow Carbon dioxide
 \rightarrow Mycelium

Both citric acid and oxalic acid were found as products of respiration, and if the fungus were supplied with citric acid, oxalic acid was produced, so that the order of decomposition must be that given above.

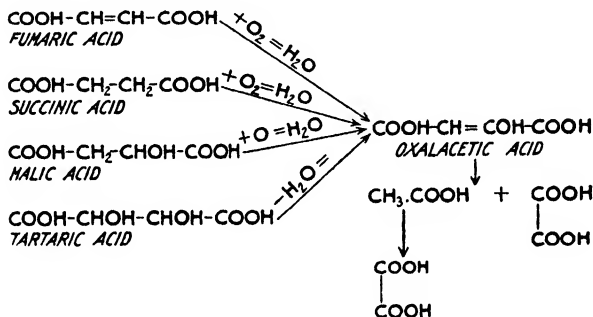
Raistrick and Clark (1919) have made a very full investigation of the formation of oxalic acid from organic acids by *A. niger*. The best growth of the fungus was found to occur when it was supplied with three-carbon acids, such as lactic and pyruvic acid, but no oxalic acid was produced. With four-carbon dibasic acids, such as succinic, fumaric, malic and tartaric acid, good yields of oxalic acid were obtained. Again, with the two-carbon acids, glycollic, glyoxylic and acetic acid, only acetic produced oxalic acid, although good growth of the mycelium was found in all three cases. When the medium was made up with ammonium butyrate and propionate no growth occurred. In all the cases investigated, the four-carbon polybasic acids gave the greatest yield of oxalic acid.

Raistrick and Clark from these results argued that the breakdown of hexose to oxalic acid takes place in the following stages :—

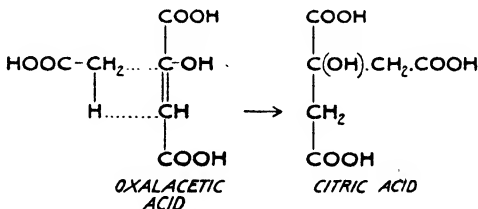


The formation of (II.) by simple dehydration is the enolic form of a polyketide, a substance possessing the $\text{CH}_2\text{—CO—}$ grouping in the molecule. The fact that free acetic acid was never found in the various media is the main drawback to the theory. But it must be remembered that *A. niger* has a very swift action on acetic acid and rapidly converts it into oxalic acid. This may account for its absence in the culture media.

The scheme suggested for the breakdown of dibasic acids to oxalic acid is by the intermediate production of oxalacetic acid (IV.) in the scheme given above:—

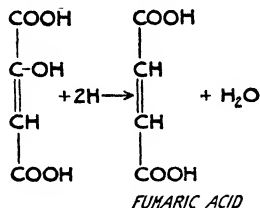


The fact that in three cases direct oxidation with oxygen is postulated and in the fourth dehydration is not a help to the theory. The production of citric acid by *A. niger* is said to be due to the addition of one molecule of acetic acid with a molecule of oxalacetic acid:—

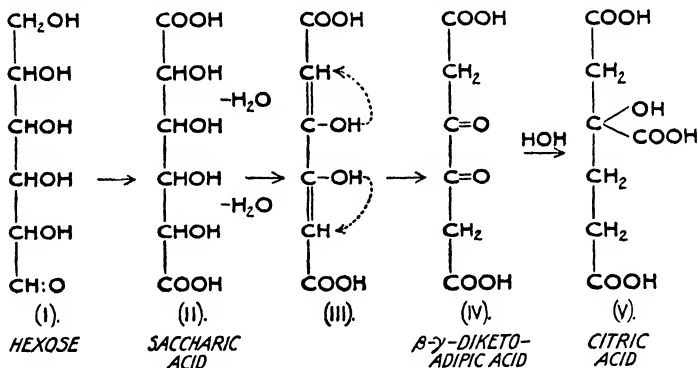


It may conveniently be mentioned at this stage that Wehmer (1918) has isolated a strain of *Aspergillus niger*, to which he has given the specific name of *A. fumaricus*, owing to its ability of forming fumaric acid from hexose. Raistrick and Clark con-

sidered that a molecule of oxalacetic acid in this case undergoes direct reduction by hydrogen, instead of further oxidation as in *A. niger* :—



Franzen and Schmitt (1925) considered that the biological formation of citric acid from hexoses by *A. niger* takes place through the intermediate formation of saccharic acid :—



Saccharic acid, however, has never been discovered as a product of mycological action.

More recently Challenger and his associates (1927) have carried out an extensive investigation of the production of oxalic acid by *A. niger*, and have evolved a complete scheme to account for the oxidation of hexose to oxalic acid. It was found that when the fungus was grown on citric and other organic acids, glyoxylic acid was produced. When it was grown on citric acid both acetone and malonic acid were discovered to be present. When cultivated on a medium with calcium acetate, glycollic, glyoxylic and oxalic acids were formed. Lastly, if *A. niger* were grown on potassium saccharate, citric acid was isolated. From

these results they considered that the series of chemical reactions involved proceed on the following lines :—

Glucose \rightarrow gluconic acid \rightarrow saccharic acid \rightarrow β - γ -diketo-adipic acid \rightarrow acetone-dicarboxylic acid \rightarrow malonic acid \rightarrow acetic acid (2 mols.) \rightarrow glycollic acid \rightarrow glyoxylic acid \rightarrow oxalic acid.

Cross and Bevan (1919) have found that in *Shorea robusta*, a forest tree of the Himalayas, large quantities of calcium oxalate are secreted in the bark as a result of respiratory processes. The position of the active oxalic acid-producing centres was not discovered.

Carbon Monoxide as a Product of Respiration.—Carbon monoxide has been discovered as a product of respiration in the pneumatocysts of the laminarian, *Nereocystis Lutkeana*. It was shown by Langdon (1917) and Langdon and Gailey (1920) that the bladders contain an atmosphere of nitrogen, oxygen and carbon monoxide, but no carbon dioxide. Over a thousand of these floats were analysed and the percentage of this gas was found to vary between 1 and 2 per cent. by volume, and the oxygen fluctuated between 15 and 25 per cent. It was only in the presence of oxygen that the carbon monoxide was produced in the bladders. If the oxygen were replaced by either hydrogen or nitrogen, the production of the gas ceased. The formation of the monoxide proceeded both by day and by night and was not formed if autolysis were allowed to set in by grinding up the tissues. The only conclusion that can be drawn from these facts is that the gas is a respiratory product. The seat of formation in the cells and the mechanism of production still remain to be discovered.

The Nature of Aerobic Respiration

According to F. F. Blackman (see Kidd, 1916), there are always two types of respiration proceeding simultaneously in the living cell. The first consists of an oxidation of carbohydrate or fat to carbon dioxide and water, to which he has given the name "floating respiration," and to the second he has applied the term "protoplasmic respiration." The latter process is considered to

supply the necessary minimum of energy for the maintenance of the activity of the cell. Moreover, there are two stages in the process, the first consists in the anaerobic splitting off of carbon dioxide and some easily oxidisable substance, and the second stage lies in the oxidation of this second substance by the oxygen of the air. Thus fundamentally considered aerobic and anaerobic respiration have the same origin, and it is only in the end stages of the process that differences arise.

This original conception has been considerably extended by F. F. Blackman (1928) and his co-workers at the Cambridge Low Temperature Station by their study of the respiration of stored apples.

Blackman and Parija (1928) found that when apples are stored they show a steady drift in respiration, which to distinguish it from the phases of adolescence and maturity is termed the senescent phase. In this senescent phase a fundamental change is found in the organisation of the tissues, which is considered to be due to the lowering of the normal organisation-resistance, so that hydrolysis proceeds at a faster rate than in the phase of maturity.

This change leads to a greater production of the effective substrate for respiration and so to an increased production of carbon dioxide. When this senescent phase has completed itself, respiration falls in the direction of zero by the natural starvation condition that is present in an isolated plant organ.

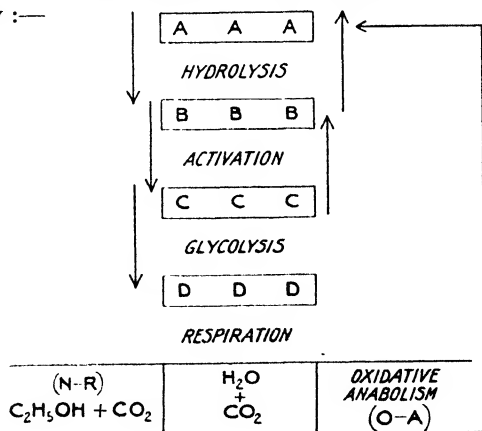
By the study of a large number of cases it was discovered that the observed respiration of an apple was due to the integration of two independent and opposed processes that were at work during senescence. One of these was a starvation drift which continuously tended to lower respiration, while the other tended to accelerate respiration by lowering the organisation resistance, expressed here by a rise of hydrolysis facility.

Parija (1928), by a continuation of these studies in respiration, discovered that prolonged exposures to nitrogen had no disturbing effect upon metabolism. When the apples were returned to air, the respiration recovered and returned to the same line of starvation drift along which it was travelling before the nitrogen was applied. The common feature, however, in all cases where the

apples were transferred to an atmosphere of nitrogen was a sharp rise in the rate of respiration. It is probable that the difference between the air condition is something other than the difference between the carbon dioxide produced by oxidation of sugar and the carbon dioxide produced by the splitting of sugar.

A survey of the respiration in nitrogen and the "air-line" respiration (*i.e.*, the respiration line in air continued to meet the respiration graph when the apples were returned to air from nitrogen) showed that the metabolic factors which determined the progress with time were different in the two cases. It was ascertained that there was a close correlation of the initial magnitude of the nitrogen-respiration with the air-line respiration, but after that the nitrogen-respiration values followed a course of their own. One clear point emerged from these investigations, namely, that the grade of starvation in air determined whether the nitrogen-respiration values lay above the air-line respiration for hundreds of hours or for only a few tens of hours.

Blackman (1928) pictured the whole drift of the metabolites involved in respiration as a drift in a system of catalysed reactions. The scheme in this system takes the form of a chain of reactions, so that the products formed by one link become the reactants of the next link. At the free end the chain becomes branched, and we find alternate fates for the reactants controlled by the supply of oxygen. A diagrammatic representation of the scheme is given below :—



A includes all substances in the apples which may function as reserves of carbohydrates and give rise by hydrolysis to free normal hexoses (B). Blackman assumed that the hexoses are not respired directly, but that a further carbohydrate stage intervenes, to which he gave the term "activation," leading to the formation of heterohexoses with the less stable type of internal ring structure. This group (C) is considered as the direct substrate of the next reaction entitled glycolysis. Glycolysis is similar in nature to the action of zymase in yeast which converts hexoses into ethyl alcohol and carbon dioxide, as well as a number of other substances, such as acetaldehyde, lactic and pyruvic acid, etc. The products of this reaction (D) are considered to be the reactants for the last stage, and here alternatives are possible, depending on the presence or absence of oxygen.

In the presence of nitrogen, group (D) proceeds quantitatively to the final products, carbon dioxide and ethyl alcohol, and these escape from the system as waste products. On the other hand, the system behaves differently in air, as well as in other concentrations of oxygen. In the first place it must be remembered that oxygen has no action on zymase activity. In air the final products of respiration are carbon dioxide and water. Again, it has been seen that the amount of carbon dioxide produced in oxygen-respiration is less than in nitrogen-respiration. In fact, it was found that the total loss of carbon was three or four times as great in nitrogen as in air. Now, no final products of (D) accumulate in the tissues during oxygen-respiration. It is, therefore, logical to assume that in air part of the group is somehow worked back into the system continuously in the presence of oxygen. There is then a call for a third reactive mechanism dealing with (D), which Blackman calls "oxidative anabolism" (O—A). This anabolic building back is specific in the presence of oxygen, but short up-grade reactions occur in these linked reactions which are held to be directly reversible. Thus (B) may be converted back to (A) by condensation, or (C) to (B) by reversion. On the other hand, (D) to (C) is held to be irreversible.

The Factors affecting Respiration

Like other physiological processes, respiration is markedly affected by internal and external factors. The chief factors which influence the rate and intensity of respiration are tabulated below :—

1. The amount of respirable material.
2. The amount of oxygen present in the air.
3. Water.
4. The amount of carbon dioxide in the air.
5. Acidity.
6. Salts.
7. Temperature.
8. Light.

The effect of a considerable number of these factors is too well known to be considered here, but important advances have been made in others in recent years, and the more relevant of these investigations will now be discussed.

The respiration of *Helianthus annuus* under field conditions has been exhaustively studied by Kidd, West and Briggs (1921). The respiration of a representative plant of a crop was determined at frequent intervals at constant temperature. From these results it was possible to determine the respiration of a mean plant of a crop at the recorded fluctuating temperatures of the field, and thus a measure was obtained of the rate of loss of dry-weight of the plant. The effect of age could also be found from these values. These investigators were able to show that a group of internal factors are also concerned in respiration which have a marked influence upon the process.

The following factors were found to be important in their effect on the rate of respiration per unit dry-weight of the plant : (i.) the concentration of respirable material ; (ii.) the effective amount of respirable cell-matter per unit dry-weight : the so-called " internal " factor for respiration ; (iii.) the concentration of oxygen ; and (iv.) the temperature.

This internal factor could be most readily determined when all

the other factors concerned were not limiting respiration. The respiration expressed as per gram weight of dry matter per hour, when measured with respiration matter in excess, with the external concentration of oxygen equal to that of the air and the temperature at 10° C., was termed the "respiratory index." It was found that there was a continual falling off in respiration as measured by this index with the advance of age; a similar falling away was discovered in the stem, leaves and flowers. The respiratory index of the stem apex also decreased with age, indicating that the respiratory index of the meristematic tissues decreased with age. This fall in the respiratory index of the meristematic tissues and leaves indicates very clearly that the total fall in respiration in the plant with age is not due to an increase in mechanical tissue, such as xylem and sclerenchyma. A close connection was found between the "internal" factor for respiration and the "internal" factor for growth.

Water.—Since water is always present in large quantities in the cells of living organisms, it is probable that it plays a large and important part in influencing the respiratory intensity. Such has been found to be the case. Bailey (1921) found that the moisture-content in large measure determines the respiratory rate of sound corn stored under uniform conditions of temperature. With increase of moisture an increase in the respiration rate was induced; with an increase of 15 to 17 per cent. in the moisture content, the respiration rate was increased by nearly 400 per cent. The respiration of corn during ripening was found to be much lower than later in the season, possibly due to a reduction in the rate of oxygen diffusion into the respiring cells and a reduction in the rate of diffusion of carbon dioxide passing out or possibly to a combination of both these processes. Cracked and broken corn showed an original respiration rate considerably higher than sound corn.

According to Jacquot and Meyer (1925), seeds of the broad bean, maize and pea-nut must absorb a certain amount of water before carbon dioxide is evolved. As the amount of water is increased in quantity, so there is a rise in the rate of respiration to a maximum, and this is then followed by a fall.

Meyer and Plantepol (1925) measured the oxygen and carbon dioxide exchange of mosses in the dark. The water-content influenced the intensity and variety of the oxidation. The respiratory co-efficient varied with the degree of imbibition of water. When imbibition was feeble, the rate became higher than unity. The carbon dioxide evolved was the result of respiration, and was not evolved from any gas dissolved in the water employed in these experiments. In dry moss plants placed *in vacuo*, as much carbon dioxide was evolved as in air. It is evident that the carbon dioxide evolved in such circumstances must have been a product of anaerobic respiration.

Richards (1927) has investigated the connection between the rate of respiration and the water-content in the higher fungi (basidiomycetes). He was able to show that with increase of water-content there was a rise in the rate of respiration. In some cases an apparent optimum amount of water was found for the respiratory rate.

Acidity.—The acidity or alkalinity of the medium may, in certain cases, influence the rate of respiration. Gustafson (1920) studied the effect of the concentration of hydrogen ions on the respiratory rate of *Penicillium crysogenum*. Variations in the *pH* value between 4 and 8 produced practically no effect on the normal rate, *i.e.*, the rate of neutrality (*pH* = 7.0). Increase of the *pH* to 8.8 caused a fall of 60 per cent. in the rate, after which it remained constant for the rest of the experiment. With decrease in the *pH* to 2.65 there was a gradual rise in the respiration followed by a fall to normal. When the *pH* was further reduced to 1.10 to 1.95 there was a preliminary rise of 20 per cent. followed by a fall below normal. The decrease in the rate found here was ascertained to be irreversible, whereas the decrease produced by raising the *pH* to 8.8 was only of a temporary character, and the respiration returned to normal when the medium was changed to *pH* 7.0. It was also found that in acid media, *i.e.*, those with a low *pH*, there was a considerable increase in the consumption of oxygen, and that the reverse held good in alkaline media.

In neutral solutions of glucose and hydrogen peroxide, Gustafson (1921) found that there is an increase in the production of

carbon dioxide by *Penicillium crysogenum* on the addition of acid, but not by the addition of alkali.

Salts.—The action of salts on respiration depends upon their nature and concentration, and the matter is further complicated by the question of antagonism. Gustafson (1920) discovered that in a 0.05 per cent. glucose medium, the respiration of *Aspergillus niger* was increased by the addition of sodium chloride in concentrations varying between 0.35 and 0.5 molar, and also by the addition of calcium chloride up to 0.5 molar. Stronger concentrations of sodium chloride (2 M.) and calcium chloride (1.25 M.) brought about a decrease in the rate, but a mixture of the two salts in the above concentrations and in the proportion of 19 c.c. of sodium chloride and 1 c.c. of calcium chloride showed antagonism. Spores of *A. niger* refused to germinate in the presence of a 0.5 M. solution of sodium chloride, whereas quite easy germination took place in the presence of a 0.5 M. solution of calcium chloride, and a mixture of the two salts exhibited antagonism.

Brooks (1920A) has found that concentrations of magnesium chloride solution up to 0.01 M. have little effect on the respiration rate of *Bacillus subtilis*. Well-marked antagonism was shown between sodium chloride, magnesium chloride and calcium chloride. Brooks (1920B) has also ascertained that low concentrations of sodium, potassium and calcium chloride have little effect on the respiratory rate of *B. subtilis*, for the rate remained constant for hours, whereas high concentrations (0.15 and 0.2 M.) of sodium and potassium chloride and calcium chloride (0.5 M.) brought about an increase in the rate. Still higher concentrations lowered the rate. The antagonism between mixtures of sodium and potassium chloride was small, but well-marked antagonism was exhibited between either sodium or potassium chloride and calcium chloride. The antagonism curve was found to show two maxima. Of the various salts described above, potassium chloride is said to have the least toxic action in high concentrations, and is also said to have the least influence on the respiratory rate.

Brooks (1921) has also shown that the respiration of bacteria is not affected by the presence of lanthanum nitrate in a concentra-

tion of 25×10^{-6} M. Smaller concentrations than this raised the respiratory rate, while higher concentrations lowered it. At a concentration of 0.8 M. the respiration was reduced to zero. In mixtures of sodium chloride and lanthanum nitrate the respiration was at a maximum when the ratio La : Na was 0.2 : 99.8. With calcium chloride and lanthanum nitrate the ratio for maximum respiration was as 8 : 2, but the results were not so marked as when sodium chloride was used.

According to Lyons (1924) phosphates have a marked influence on the respiration rate of *Elodea canadensis*. Plants grown in neutral phosphate solution of concentrations 0.021, 0.085 and 0.106 M. showed a depression in respiration followed by an acceleration. Even with concentrations as high as 0.17 M. an acceleration in the respiratory rate was shown. Lyons considered that the phosphate accelerates the aerobic phase of respiration, for when the apparatus was filled with hydrogen no carbon dioxide was produced. The matter, however, is complex. In wheat seedlings, the anaerobic phase is affected as well as the aerobic. In experiments conducted over a long period, it was discovered that the preliminary acceleration gradually lessened and then sharply rose to a new maximum even greater than the first, and this second steep rise was followed by a rapid fall. Plasmolysis tests revealed that the cells were dead when the curve had reached the first maximum, so that evidently there was a considerable rise in respiration after death.

Cook (1926) has tried the action of the chlorides of copper and mercury, as well as silver nitrate on the respiration rate of *Aspergillus niger*. The respiration was reduced in amount and the speed of the toxic action of these salts was found to be a constant power of their concentration.

Light.—It has been known for some time that light, besides its indirect effect of starting the photosynthetic mechanism, has a direct effect on respiration. This direct effect has usually been ascribed to the ionising action of the light on air. Using a "pure line" of barley, Middleton (1927) investigated the rate of respiration in normal and ionised air. The ionising agent employed was the radio-active element polonium. The polonium

was applied for an hour, and then intermitted for an hour, and then again applied for another hour. The respiration increased during the period of application. The maximum increase registered was 29 ± 5.62 per cent. during the second hour. The acceleration varied with the degree of ionisation. If the ionisation were 20,000 times that of normal air, increase in the respiration occurred from both applications of the polonium. If the degree of ionisation were 100,000 times that of ordinary air, there was only a significant increase at the second application. Still higher degrees of ionisation gave barely significant values of increase. Similarly, Whimster (1927) has observed that in the presence of polonium which gave a degree of ionisation varying between 7.28×10^2 to 3.64×10^2 of that of normal air, the leaves of *Pelargonium zonale* gave as high a percentage increase as 85.7 ± 7.1 in the respiration rate. A curious after-effect was noticed in the succeeding two hours after the removal of the polonium, when the percentage increase rose to 28.0 ± 7.1 over the control period. The effect on respiration was found to be due to the ions themselves, and not to the presence of ozone.

Anæsthetics

A large amount of the older work on this aspect of respiration went to prove that the action of anæsthetics was to cause an increase in the intensity of the respiration and that this increase was followed by a decrease. Gustafson (1919) found that formaldehyde, ether and acetone caused an increase in the rate of respiration of *Aspergillus niger*, and this increase was followed by a decrease. A 0.5 per cent. solution of caffeine also brought about a similar result. Brooks (1919) found a similar state of affairs for *Bacillus subtilis*. Here there was an increase followed by a decrease in respiration in the presence of ether. If a 0.85 per cent. solution of sodium chloride were added, antagonism was shown between the ether and the chloride. In high (3.65 to 7.3 per cent.) and low (0.37 to 1.1 per cent.) concentrations of ether, the anæsthetic proved to be toxic to the bacterium, but in intermediate concentrations it acted as a stimulant. Thomas (1919)

has ascertained that ether brings about an increase followed by a decrease in the respiratory rate of wheat seedlings. Too long exposure, over thirty minutes, results in death. Irving (1919) showed that the petals of *Salvia* in the presence of high concentrations of ether showed an increased oxygen consumption and output of carbon dioxide, and at the same time there was a decrease in the acidity of the cell contents.

Haas (1919) studied the action of anaesthetics on the respiration of *Laminaria*. Small doses were without effect, while larger doses gave a prolonged effect, and with still larger doses there was a slight increase followed by a decrease to zero. Haas also studied the action of anaesthetics on the respiration of *Laminaria* after death. The killed tissue was treated with such substances as ether, acetone, ethyl bromide, alcohol and formaldehyde. Using the pH method of measuring respiration (see Osterhout, 1919) and electrical conductivity of the tissues to determine their death-point (the latter was arbitrarily

fixed as 15 per cent. below normal conductivity), it was found that there was no particular fall in carbon dioxide output at the death-point, and it might even be higher than in the living tissues.

A fundamental contribution to the action of anaesthetics on respiration has been made by Smith (1921, 1924). Working with a "pure line" of wheat and using the pH method of measuring

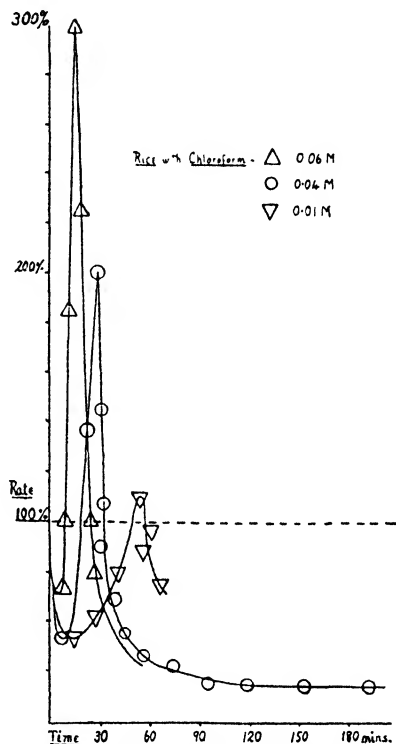
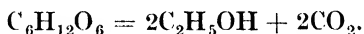


FIG. 41.—Respiration of rice with different concentrations of chloroform. (After Smith. *Annals Bot.*)

respiration with the indicator phenol red, Smith discovered that there was an initial decrease in the rate, succeeded by a rise, and this in turn was followed by a decrease to the normal value in distilled water (Fig. 41). Smith made the claim that the action of anæsthetics is to decrease the permeability of the plasma-membrane of the cell to carbon dioxide. The carbon dioxide is held within the cell in the early stages of the process, and it is only later that it can make its way out, so that the subsequent increase shown in the curve may be partly or wholly illusory.

Anaerobic Respiration

In the absence of free oxygen, plants are able to respire for a time, and are said to undergo anaerobic respiration. This is also sometimes spoken of as intramolecular respiration, which is not a particularly happy term. In anaerobic respiration the main products of the reaction are ethyl alcohol and carbon dioxide, and the equation showing the beginning and end products may be written :—



From the point of view of the plant, anaerobic respiration is an extravagant and wasteful process, inasmuch as the ethyl alcohol produced is a waste product, incapable under the experimental conditions of undergoing further oxidation.

Fermentation.—In its main features the fermentation of sugars by yeasts is similar to the anaerobic respiration of higher plants. It is not exactly similar, for Kostychev some years ago found that certain of the higher fungi, such as species of *Agaricus*, failed to form alcohol in the absence of free oxygen. Carbon dioxide, however, was evolved in considerable quantity. When mannite was added to the press-juice it disappeared without the evolution of carbon dioxide. It was possible that the substance producing carbon dioxide here was an intermediate product of oxidation and split off the gas by hydrolysis.

The production of ethyl alcohol by the yeasts is a process of very considerable industrial importance, and its discovery is lost in the mists of antiquity. Although the main products of the

reaction are ethyl alcohol and carbon dioxide, other substances are also produced in varying amounts, such as acetaldehyde, pyruvic acid and glycerol, as well as the two higher alcohols, isobutyl carbinol and secondary butyl carbinol, which are usually spoken of in commerce as amyl alcohol and form the constituents of fusel oil. The presence of fusel oil is especially pronounced in the cheap spirits produced from potatoes.

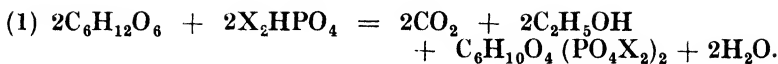
The hexoses are more suitable for fermentation than the disaccharides, as the latter have to undergo a preliminary hydrolysis before they are converted into alcohol and carbon dioxide. Alcoholic fermentation is an enzymic process and the enzyme involved is known as zymase.

It was early shown by Harden and Young that the zymase obtained from expressed yeast juice is separable into two parts: the enzyme and co-enzyme. The nature of the latter is at present unknown. According to Neuberg and Sandberg (1920) the co-enzyme plays the part of hydrogen acceptor. They were able to show that aldehydes, ketonic acids, nitro-bodies and disulphides can all serve as co-enzymes. Euler and Myrbäck (1924) found that the activity of the co-enzyme could be raised by successive precipitation with lead acetate or even silico-tungstic acid.

✓ The Influence of Phosphate in Fermentation

The addition of alkaline phosphate to the fermenting liquors markedly increases the rate of the reaction, and it was shown by Harden and Young that the increase in rate could be as high as twenty times the original value. In time the rate again falls to the original value and may be increased by a second addition of phosphate.

It was found by Harden and Young that if the solution were boiled the moment the velocity had fallen to the initial value, the added phosphate could no longer be precipitated by uranium acetate, and that it was in organic union with the hexose as a hexosediphosphate. These workers, therefore, considered that the fermentation takes place in stages and the first part of the reaction consists in the formation of a hexosediphosphate:—



Under the influence of water the hexosediphosphate undergoes hydrolysis to hexose and phosphate once more :—



The matter has since been shown to be very much more complex than was originally considered. Robison (1922) showed that if either glucose or fructose were fermented by yeast that, in addition to the hexosediphosphate, a hexosemonophosphate was also formed. Harden and Henley (1927) therefore reinvestigated the problem in the light of Robison's discovery. The original equation formulated by Harden and Young was based on the ratio CO_2/P (total esterified) = 0.9. Harden and Henley found that about 10 per cent. of the phosphorus is esterified without the evolution of carbon dioxide. They considered the product to be a hexosemonophosphate, and that the small deviation from the theoretical ratio of $CO_2/\text{hexosediphosphate} = 2$ (which they found to be 2.38 on redetermination) required by the original equation could be accounted for by the partial hydrolysis of the hexosediphosphate. This again will not meet the case. Harden and Henley (1929) found that on fermentating glucose and fructose in the presence of phosphate with *dried* yeast, the ratio of extra carbon dioxide evolved to phosphorus esterified tended to be slightly higher than unity. However, with maceration extract of yeast-juice, the values tended to be lower than unity. Further, with the dried yeast, the ratio of hexosediphosphate to hexosemonophosphate formed in the course of the fermentation was generally high, the product in certain extreme cases being almost entirely diphosphate or monophosphate and the proportions of these substances varied between 96 per cent. of the former to 86 per cent. of the latter. Yet, in spite of the very great variations shown in the amounts of these products produced, the molecular ratio of CO_2/P (total esterified) was still approximately unity.

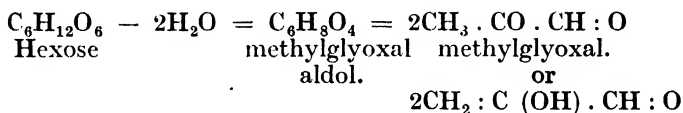
These fresh results make it impossible to express the results by means of the original equation formulated by Harden and Young, and Harden and Henley have now abandoned the suggestion

made in their first paper to account for these large deviations from the theoretical ratio.

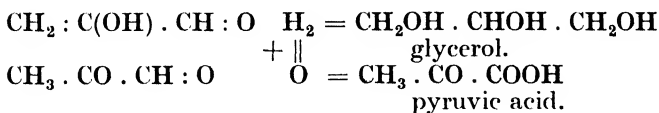
The Chemical Mechanism of Alcoholic Fermentation

Neuberg and his co-workers have carried out much valuable work in the elucidation of the chemical mechanism of alcoholic fermentation. In the scheme originally promulgated by Neuberg and Kerb (1913) methylglyoxal was the starting-point for the later stages of the reaction. The number of oxidations and reductions involved were all assumed to be brought about by a series of Cannizzaro reactions of the various aldehydes produced as intermediate products.

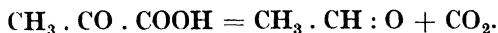
The first stage of the reaction, according to these investigators, consists in the splitting of the sugar into two molecules of methylglyoxal (pyruvic aldehyde), the reaction taking place in two steps :—



From the two molecules of methylglyoxal by simultaneous oxidation and reduction (Cannizzaro reaction) under the influence of water, pyruvic acid and glycerol are produced :—

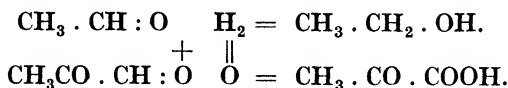


By the action of the enzyme carboxylase, which is present in the yeast cells, the pyruvic acid is decomposed into carbon dioxide and acetaldehyde :—



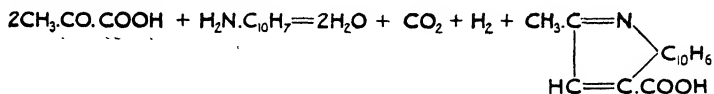
Again under the influence of water, a second Cannizzaro reaction takes place, and the acetaldehyde is reduced to ethyl alcohol with

the simultaneous oxidation of a molecule of methylglyoxal to pyruvic acid :—



The pyruvic acid thus formed is converted into acetaldehyde and carbon dioxide.

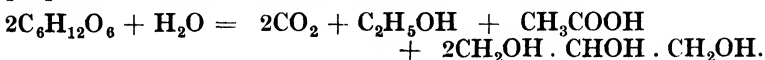
Grab (1921) demonstrated the production of pyruvic acid in alcoholic fermentation by means of the condensation product formed between this acid and β -naphthyamine to give α -methyl β -naphocinchoninic acid :—



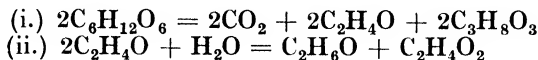
Neuberg and Reinfurth (1918, 1919, 1920), as well as Connstein and Lüdecke (1919) independently, have obtained considerable evidence for the confirmation of this theory of alcoholic fermentation.

Neuberg and his co-workers have investigated the course of fermentation under two different conditions : (a) in the presence of alkaline salts ; (b) in the presence of sodium sulphite.

Fermentation in the Presence of Alkaline Salts.—It was found by Neuberg and his co-workers that the presence of alkaline salts, such as ammonium carbonate and other soluble carbonates and phosphates, markedly influenced the course of fermentation. Provided that the salt were added after the fermentation had become well established, it was found that increased amounts of acetaldehyde, acetic acid and glycerol were produced. It was further discovered that the aldehyde produced was exactly equivalent to the glycerol formed, and Neuberg and Hirsch (1919 ; see also Neuberg, Hirsch and Reinfurth, 1920 ; Neuberg and Ursam, 1920) have shown that this equivalence persisted through the whole course of the fermentation. Neuberg therefore proposed the following equation for this reaction :—

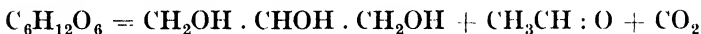


or the reaction may be regarded as proceeding in two steps :—

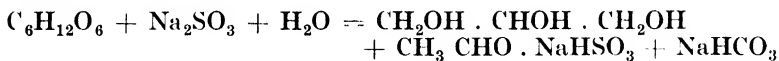


The acetaldehyde produced in the reactions is considered to undergo the Cannizzaro reaction giving equimolecular proportions of ethyl alcohol and acetic acid, as in equation (ii.).

Fermentation in the Presence of Sodium Sulphite.—When sodium sulphite was added to the fermenting liquors the amount of alcohol and carbon dioxide diminished. It was also found that the aldehyde produced was exactly equivalent to the glycerol formed (see Neuberg and Hirsch, 1919). Thus the addition of sulphite to the fermenting mixture increases the yield of glycerol. This fact was commercially exploited by the Germans during the Great War for the production of glycerol in large amounts for the manufacture of explosives without fats; animal fats having been needed for food. Neuberg proposed the following equation to express this type of fermentation :—



or since sodium sulphite is added to the fermenting liquors the equation can be written :—



In actual practice it was found that some of the sugar underwent normal fermentation, so that the final result should be expressed by a combination of the equation given above and that usually written for alcoholic fermentation :—



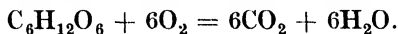
It should be mentioned here that since pyruvic acid, $\text{CH}_3\text{CO} \cdot \text{COOH}$ also possesses a ketonic group, it, like acetaldehyde, can also form a bisulphite compound : $\text{CH}_3 \cdot \text{C}(\text{OH}) \cdot \text{SO}_3\text{Na} \cdot \text{COOH}$. Neuberg found that this compound was readily fermented by yeasts, whereas the acetaldehyde bisulphite was not. Thus, only acetaldehyde and not pyruvic acid is fixed in the course of fermentation by the addition of sodium sulphite.

There is now a large amount of evidence to show that acetaldehyde is also a product of anaerobic respiration in the higher plants. Klein and Pirschle (1926) found that acetaldehyde was produced in strongly respiring organs such as buds and seedlings. Similarly, Bodnár, Szepessy and Ferenezzy (1925) discovered that peas grown under anaerobic conditions also produce acetaldehyde. If the acetaldehyde were fixed with sodium sulphite, the amount of alcohol and carbon dioxide evolved was reduced in amount. Neuberg and Gottschalk (1925) have also discovered that acetaldehyde is a product of anaerobic respiration, and if calcium, instead of sodium, sulphite were added to the mixture, the quantity of aldehyde was increased and the amount of alcohol reduced.

Thomas (1925) has investigated the anaerobic respiration of stored apples. Ethyl alcohol and acetaldehyde were both produced in the course of the experiment, though neither formed the intermediate or end-products of normal respiration in the presence of oxygen. A curious point discovered was that carbon dioxide, even in the presence of oxygen, could cause carbon dioxide zymosis, *i.e.*, respiration was of the anaerobic type. In such circumstances, the ratio of alcohol to aldehyde was 2 : 1, whereas in anaerobic respiration the ratio was 50 : 1. Hence the presence of a mixture of carbon dioxide and oxygen may be more injurious to the fruits than the entire absence of oxygen.

The Oxidation Mechanism of the Cell

Respiration is usually defined as the intake of oxygen and the excretion of carbon dioxide from the physiological combustion of carbohydrate and fat, and is expressed by the equation :—



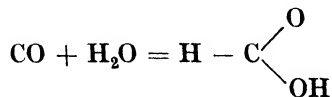
It must be remembered that this equation merely states the beginning and end products of a complex series of reactions. Glucose in ordinary oxygen or air is not appreciably oxidised, and it is therefore obvious that in the living cell some mechanism must be present for activating the oxygen to carry out the oxidation of this carbohydrate at the ordinary temperature.

The principal function of respiration is to supply the living organism with sufficient energy to carry out its vital activities, and any oxidation processes occurring in the cell which release usable energy may be classed as respiration. Adopting this wider definition of respiration, a number of different oxidation mechanisms have recently been discovered to be present in the cells of plants and animals, and their nature will now be considered.

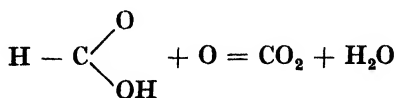
The chemical definition of oxidation is more comprehensive than the simple addition of oxygen to a substance. Oxidation, considered from the chemical standpoint, is the addition of oxygen or any electro-negative element to a substance, or the subtraction of hydrogen or any electro-positive element. Similarly, reduction is defined as the subtraction of oxygen or any electro-negative element or the addition of hydrogen or any electro-positive element to a substance.

The formation of quinones from phenols is an oxidation reaction involving the subtraction of hydrogen. This type of oxidation seems now to be of common occurrence in living cells. It needs the presence of some substance capable of taking up the hydrogen, which is spoken of as the hydrogen acceptor.

Oxidation is a complex process. Even the oxidation of carbon monoxide to carbon dioxide needs the presence of a minute trace of water, and combination will not take place in the presence of pure, dry oxygen. Wieland has made the suggestion that this reaction takes place in stages. The first stage is the combination of the carbon monoxide and water to give formic acid :—



Under the influence of oxygen the formic acid is oxidised to carbon dioxide and water :—



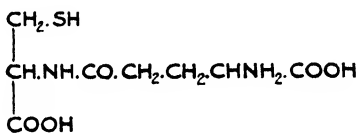
In the second stage of the reaction, the oxygen plays the rôle of hydrogen acceptor. The number of examples of oxidations brought about by the removal of hydrogen may be multiplied indefinitely, and, according to Wieland, enzymic oxidation reactions are brought about by the activation of the hydrogen of the substrate and the activated hydrogen is then removed by a suitable hydrogen acceptor, such as atmospheric oxygen. He explained such phenomena as the Schardinger reaction, which is used for distinguishing boiled from unboiled milk, as due to the presence of an enzyme which plays the part of a hydrogen acceptor. In the Schardinger reaction, milk is treated with a drop of acetaldehyde and methylene blue. Should the milk have been previously boiled, the methylene blue is not decolorised to the leuco-base on warming; if the milk has not been boiled, the dye is reduced to the leuco-base. Wieland considered an enzyme to be present, to which he gave the name dehydrase or reductase. This enzyme dehydrogenates the aldehyde hydrate first formed and the methylene blue plays the part of hydrogen acceptor.

In physiological experiments, the dye, methylene blue, is used to detect these oxidation-reduction reactions. In the presence of a hydrogen donator, the dye is reduced to the colourless leuco-base, and in the presence of a hydrogen acceptor it is oxidised to the dye once more.

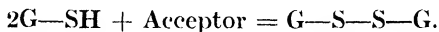
Thunberg (1920) removed by washing, the reducing substance (glutathione, see below) in frog's muscle which reduces methylene blue. He found that on addition of a variety of organic compounds, *e.g.*, succinic acid, the latter brought about the reduction of the dye, whereas others, such as propionic acid, which does not yield its hydrogen readily, did not. Thunberg considered that the methylene blue was reduced by the giving up of hydrogen by some donator, and that this enzyme was transported by an enzyme to the acceptor, in this case methylene blue. From his experiments, Thunberg suggested that hydrogen is the primary fuel of the cell.

Hopkins (1921, 1923) claimed to have isolated from yeast a dipeptide to which he gave the name glutathione which could act either as a hydrogen acceptor or hydrogen donator. On hydro-

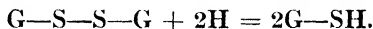
lysis with hydrochloric acid it gave cystein and glutamic acid and Hopkins assigned to it the following constitution :—



Thus in the presence of a hydrogen acceptor the following reaction was considered to take place :—



Whereas in the presence of a hydrogen donator, the oxidised glutathione (G—S—S—G) acted as a hydrogen acceptor :—



In 1925 Stewart the Tunncliffe published an account of the synthesis of this substance and the synthetic product was alleged to resemble in all respects, including optical activity, the natural product. As a result of this synthesis all doubts were set at rest with regard to the constitution of this compound and a number of publications appeared showing the importance of glutathione in cell oxidations.

Hopkins and Dixon (1922) found that freshly washed muscle does not reduce methylene blue, but on the addition of glutathione in a solution suitably buffered, the power of reducing the dye is restored and enables the system to respire. The washing of the tissue removed the peptide. If the glutathione and washed muscle were heated to 100° C., reduction and respiration still took place. It was, therefore, thought that the glutathione was acting in the tissue with some thermostable constituent to form a simple and highly efficient autoxidisable system.

Hopkins (1925) discovered that in acid systems of *pH* 3.0 to 4.5, glutathione facilitated the oxidation of unsaturated fatty acids and lecithin. In neutral or alkaline systems (*pH* 7.4 to 7.6), the whole nature of the process seemed to be different. While the sulphydryl (—SH) group of the glutathione is being oxidised, the

fatty acids are simultaneously oxidised in such a manner that there is an equal division of oxygen between the two. Hopkins also considered that the oxidation of certain proteins by glutathione (either $G-SH$ or $G-S-S-G$) only occurs if the proteins themselves display sulphhydryl groups. In the case of the water-extractable proteins of muscle tissue, which possess this group, oxidation will only take place in neutral or alkaline media and not if the medium be acid. The sulphhydryl group of the protein is oxidised and the total amount of oxygen consumed is ten times the equivalent of the sulphhydryl group. The protein can be reduced, when the sulphhydryl group reappears and with the glutathione a further uptake of oxygen becomes possible, and this uptake is again greatly in excess of the oxygen equivalent of the sulphhydryl group.

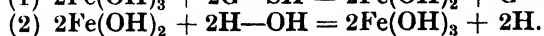
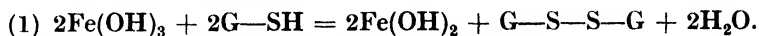
Allott (1926) found that the oxidation of fats is by no means so simple as pictured by Hopkins. Different samples of fats give different results and similar values to those of Hopkins can only be obtained with certain conditions of the oil. For example, the "double-uptake" of oxygen with fatty acids at pH 7.5 seems to depend upon the lack of spontaneous activity of the fatty acid, and the iron content of the system, and very possibly other factors may be concerned as well.

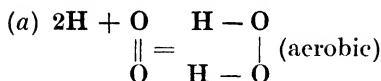
It has been known for a number of years that potassium cyanide in minute concentrations can stop all oxidations in living cells without effecting any lasting injury, and the respiratory activity is recovered on simply washing with water. Warburg (1914) found that iron was highly important in the respiratory activity of sea-urchin eggs, and that in the absence of iron, respiration came to a standstill. It is therefore probable that the cyanide combines with the iron in the cell and causes the various oxidation processes that are taking place to cease. Since cyanide and iron combine *in vitro* to give complex salts of the type of the ferro- and ferricyanides, which are stable bodies, it is difficult to ascribe any explanation of the reverse process brought about by the mere washing of the tissues.

According to Warburg, structure plays an important part in the activity of the cell; in that suitable surfaces are presented for the

activity of catalysts taking part in cell reactions. To illustrate this view, he has devised his so-called "charcoal-model." He found that if cystine and other amino-acids are shaken with blood charcoal at the ordinary temperature, oxygen is absorbed, and the acids are oxidised to ammonia and carbon dioxide. Iron is found to play an important rôle in these reactions, and narcotics act on the "model" in much the same way as in living cells. Warburg (1923) imagined the cell to be composed of a mosaic of areas of iron and no iron. The cell oxidations are considered to take place on the iron-bearing areas. He has made a number of experiments with blood charcoal containing varying amounts of iron which give strong support to his views. He considered that the function of the iron is that of oxygen carrier or oxygen activator, and that ordinary molecular oxygen is quite unable to act as a hydrogen acceptor unless it is first activated by iron.

Harrison (1924) found that the oxidation of glutathione was markedly accelerated by the presence of small amounts of iron. The iron acted in a catalytic capacity, and potassium cyanide put an end to the reaction. In a later series of experiments, Harrison (1927) was able to carry the matter a stage further, and showed that cyanide affected both the aerobic and the anaerobic phases of the oxidation of glutathione. The anaerobic experiments were carried out in nitrogen. In addition, he discovered that iron could accelerate both the anaerobic and the aerobic oxidation of glutathione. Such being the case, it is clear that the iron is not acting in the capacity of an oxygen carrier or activator. Harrison made the suggestion that the function of the iron is that of an intermediary catalyst, and that it is alternately oxidised and reduced. Ferric iron is reduced by the sulphydryl group, and the ferrous salt thus produced reduces the hydrogen acceptor present, which may be methylene blue in the anaerobic and oxygen in the aerobic experiments. In the latter case, hydrogen peroxide will be produced and the iron is simultaneously oxidised back to the ferric state. The ferric iron is then reduced by a further quantity of glutathione:—





Claims have also been made that glutathione was present in plant tissues. Kozlowski (1926) found non-protein cystein in a number of plant tissues, and especially in the pea. He considered it highly probable that it may be bound to such a substance as glutamic acid giving a peptide such as glutathione, and that a simple autoxidisable system also exists in plant cells.

As has already been stated, from the synthesis of glutathione and the alleged similarity of this compound with the natural product, the validity of this work on the importance of glutathione in the various cellular oxidations described above was accepted in its entirety in this country. Nevertheless, Hunter and Eagles (1927), in America, made the persistent claim that the glutathione they were able to isolate from liver, yeast and blood, by the method described by Hopkins, always contained less than the theoretical percentage of sulphur required by diglutaminyleysteine (9.73 per cent. as against 12.85 per cent.), and although they found both cystein and glutamic acid to be present, they considered from their analytical findings that something additional was also present as well. As a result of this work, Hopkins was led to re-investigate the constitution of glutathione and he has now shown it to be tripeptide of cystein, glutamic acid and glycine, and not a dipeptide of glutamic acid and cystein. The product was obtained in the crystalline condition by means of the copper salt. The remarkable similarity of the natural with the synthetic product obtained by Stewart and Tunnicliffe was considered to be a coincidence. It will be remembered, however, that the optical activity was also stated to be similar in both cases.

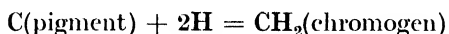
In a brief note, Dixon and Meldrum (1929) have shown that the new crystalline product does not resemble in its reactions the natural product first isolated. They have repeated the experiments of Dixon and Hopkins on protein oxidation with this crystalline product. They found differences in behaviour which have an

important bearing on the glutathione problem. It was discovered, for example, that pure crystalline glutathione was quite unable to catalyse the oxidations of proteins of thermostable muscle preparations, although the fact was confirmed that the impure product was able to do so. The addition of pure glutathione produced no oxygen uptake. The addition of a small amount of impure glutathione, on the other hand, rapidly produced oxygen removal. Like the impure product, the crystalline glutathione was found to be autoxidisable, owing to the presence of minute amounts of catalytic metals. But so far from catalysing the oxidation of proteins, the addition of muscle preparation inhibited its own autoxidation. The addition of a little impure glutathione, however, produced the desired result.

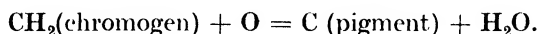
It is thus evident that though the oxidation of glutathione depends on the presence of metal catalysts, the addition of the latter alone is not in itself sufficient and the simultaneous presence of some other additional factor is also necessary. This factor, which is present in impure glutathione, is not present to any great extent in the crystalline product, and is removed by thermostable muscle preparation, and thus stabilises the glutathione. Dixon and Meldrum have suggested that it perhaps acts as a catalytically active complex with the metals that are present.

It will be necessary at this stage to consider briefly Palladin's views on the respiration mechanism of plants. He considered that certain chromogens, to which he gave the name "respiratory chromogens," were widely distributed in the plant world. To obtain these chromogens all that is necessary is to boil the tissues with water and filter the liquid. Peroxidase (see below) and hydrogen peroxide are then added, when a red colour is developed by the formation of the respiration pigment from the oxidation of the chromogen. This, by further oxidation, is converted into a black or violet-black substance. Palladin supposed that respiratory chromogens were present in the form of pro-chromogens, which were in the nature of glucosides, and these pro-chromogens were oxidised by the enzymes present in the cells to chromogens. By forming water, chromogens remove hydrogen produced in

respiration. Thus, if C represent the respiration pigment, the following reaction will take place :—



By the action of oxidase (see below) chromogen will now absorb oxygen from the air and form the pigment and water once more :—



Haas and Hill (1925A, 1925B, 1926) have been able to extract a colourless chromogen from the leaves of *Mercurialis perennis*, which shows a marked avidity for oxygen. To this substance they have given the name hermidon. Hermidon is capable of undergoing oxidation in two stages, yielding first a fugitive blue compound, cyanohermidon, and in the second stage, a stable yellow pigment, chrysohermidon. The volume of oxygen fixed at each stage of the oxidation is the same. The reaction is reversible. When treated with sodium hydrosulphite, the blue cyanohermidon is reduced to hermidon, while on shaking with air the colour is restored. The reduction of the yellow compound can only be brought about by the aid of fairly drastic reducing agents, such as the aluminium-mercury couple, which may also be used for the reduction of the cyanohermidon. It was also discovered that the leaves of the plant, soaked in a vessel containing cyanohermidon, reduces the latter to hermidon, but fails to reduce chrysohermidon.

Haas and Hill suggested that hermidon plays a part in the respiration of *M. perennis*, since it is most abundant when the respiration of this plant is at its highest in spring and summer. These authors considered that the hermidon suffers oxidation to cyanohermidon and the cyanohermidon is reduced to hermidon once more, losing its oxygen to some acceptor or reducing agent which may be of the nature of a metabolite. Cannon (1926) has studied the electrode potentials of the system hermidon-cyanohermidon over a wide range of pH (2.0 to 8.0), and the system cyanohermidon-chrysohermidon over the range of pH 7.0 to 8.0. The second system was found to be reversible only in the neighbourhood of neutrality, as the line-oxidant was subject to an

irreversible non-oxidative change, the velocity of which was a function of the hydrogen-ion concentration.

Roach (1925) has also found a blue pigment in slightly sprouted potatoes which had been placed overnight in an atmosphere of carbon dioxide in a vacuum desiccator and later pulped in an atmosphere of this gas. The filtrate from the pulp was of an opalescent blue colour tinged with green. On the admission of air, the blue colour became more intense, and with still further exposure it changed through various shades of green to light yellow. From the yellow, still further colour changes occurred, till finally a brown-black was formed. The blue shade was discharged on the addition of sodium hydrosulphite. It would seem that the potato, like *Mercurialis perennis*, contains a compound resembling hermidon, or this compound may even be generically related to hermidon.

Quastel (1926), from his studies on resting bacteria, has brought forward an interpretation of oxidations and reductions *in vivo* which embraces the views of Wieland, Thunberg and others. The bacterium employed was *Bacillus coli*. Of 103 substances tested, 56 were found to be hydrogen donators or acceptors. If enzymes were responsible for the transport of hydrogen from donator to acceptor, it is in the highest degree improbable that there are 56 specific enzymes dealing with one type of phenomenon; moreover, all attempts to isolate such enzymes have failed. Even were enzymes responsible, the actual mechanism of activation still remains to be elucidated. The problem of determining the mechanism, therefore, resolves itself into three separate problems: (1) determination of the site of activation of a substrate molecule; (2) determination of mechanism of activation of this molecule; and (3) determination of process of oxidation after activation has occurred.

From a consideration of the experimental data, Quastel considered that the site of oxidation of the substrate molecule is at the cell surface. In all growing organisms, we have a series of co-ordinated chemical reactions taking place. If the reactions were not co-ordinated, there would be no growth or other vital activity. The ordered behaviour of chromosomes shows this to

be so. In other words, an ordered series of events must be taking place in the cell.

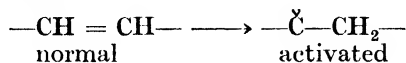
The work of Hardy, Langmuir and others has shown that membranes have a definite structure. The molecules composing the membrane are definitely orientated with regard to one another in a particular way dependent upon their chemical nature and the character of the phases on either side of the membrane. From its complex nature it cannot be expected that the membrane possesses a homogeneous character or even shows symmetry. Certain parts would be occupied by certain molecules or groups of molecules, and other parts by other molecules or groups of molecules. It is probable that there is an irregular distribution of such groups, *i.e.*, they have a geography.

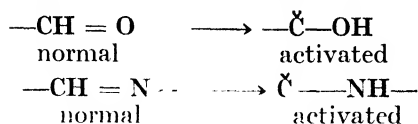
Now, associated with certain groups or molecules orientated in the membrane, there will exist electrical fields, the nature of which will depend on the nature of the groups. Some will be very powerful and others weak, *i.e.*, they will show varying intensity.

Mechanism of Activation.—If the effect of an external electric field be considered upon an unsaturated linkage, say $C_2 = C_1$, the octets of electrons round the carbon atoms, C_1 and C_2 , will have four in common. If the external electric field act from left to right on the molecule, some of the electrons held in common may be so far displaced from C_2 to C_1 that they can no longer be considered as being held in common with C_2 . If two were to go, then C_1 has a complete octet and C_2 is reduced to a sextet. Thus C_1 has become saturated, and C_2 unsaturated and chemically more active. The same force which alters the electrons will also alter the relative positions of the protons (H-ions), thus in :—



the polarising field upon the double bond will cause a shift of proton to C_2 or *vice versa*, the extent of the shift depending on the strength of the external field. The greater the shift, the more unsaturated and chemically active will one end of the bond become. The following equations represent the change after maximum activation :—





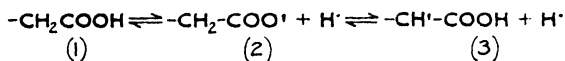
A second factor that must now be considered is that not only will there be the external electric field, but the electrical condition of the molecule must also be taken into account in determining the actual shift of the proton. It has been shown by Thomson that the electrical effect of replacing an atom of hydrogen by another atom or radical may be represented by the introduction of an electric doublet at the hydrogen atom. So that if the radical form a system requiring an electron to complete the octet, the positive end of the doublet will be towards the molecule with which the radical is combined. Thus, if a radical replaces a hydrogen atom combined to a carbon atom, the positive end of the doublet will be towards the carbon and the electrical effect at the carbon atom will be to drive away positive and attract negative electricity. The reverse effect holds with a radical in which there is one electron over, after providing for complete octets. Take, for example, the structure $\text{A—CH} = \text{CH—X}$, where A is assumed to have no directive influence on the movement of the proton. If maximum activation has taken place, the following results can occur :—

| X | NORMAL FORM OF MOLECULE | ACTIVE FORM OF MOLECULE |
|------------------------|--|---|
| —COOH | $\text{A—CH}=\text{CH—COOH}$ | $\text{A—}\overset{\cdot\cdot}{\text{C}}\text{—CH}_2\text{—COOH}$ |
| —CH ₃ | $\text{A—CH}=\text{CH—CH}_3$ | $\text{A—CH}_2\overset{\cdot\cdot}{\text{C}}\text{—CH}_3$ |
| —CH ₂ ·COOH | $\text{A—CH}=\text{CH—CH}_2\text{·COOH}$ | $\text{A—CH}_2\overset{\cdot\cdot}{\text{C}}\text{—CH}_2\text{·COOH}$ |

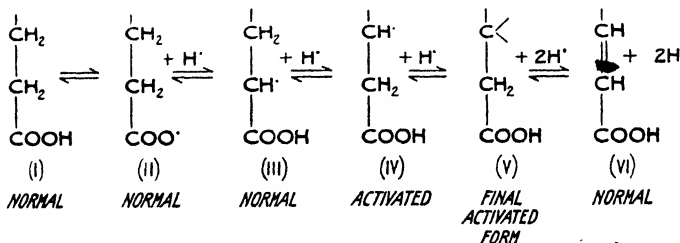
The effect of the carboxyl group at X is to produce an electric field at the α -carbon atom which attracts positive electricity. It will also affect the β -carbon atom to some extent, and may even render this more liable to attract positive electricity than it was before the introduction of the carboxyl group. The field at the α -carbon atom, however, is very much stronger, and there will be a shift of proton from the β - to the α -carbon atom. The β -carbon atom will be now unsaturated and the α -atom saturated. Hence,

when oxidation occurs *after* activation, an asymmetrical oxidation is impossible, and only β -oxidation will take place. When the methyl radical is in position X there will be a passage of proton from α - to the β -carbon atom after activation and α -oxidation will occur.

Saturated Bond Oxidation.—In the case of the oxidation of saturated compounds, different conditions arise. Take, for example, a compound of the type $-\text{CH}_2-\text{CH}_2-\text{COOH}$. Here the condition of the carboxyl group has to be considered in detail. The charged carboxyl group has an effect on the α -carbon atom, the hydrogen will be rendered more mobile and will oscillate between the α -carbon atom and one of the oxygen atoms of the carboxyl group. Thus the normal forms of the structure, $-\text{CH}_2 \cdot \text{COOH}$, will be represented by the equilibria :—



The movement of the α -H(proton) is more likely in a saturated than in an unsaturated structure, *i.e.*, in $-\text{CH}_2 \cdot \text{COOH}$ than in $=\text{CH} \cdot \text{COOH}$; for in the latter the attractive effect of the α -carbon atom for positive electricity will oppose the attractive effect of the oxygen of the carboxyl group. If, however, there be attached to the α -carbon atom, a group which has the effect of repelling positive electricity at this atom, a shift or movement of hydrogen will take place, so that the activation of a saturated group such as $-\text{CH}_2-\text{CH}_2 \cdot \text{COOH}$ will proceed as follows :—



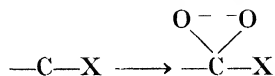
On activating (III.) proton will move from the β - to the α -carbon atom, *i.e.*, towards the atom with the strongest positive electricity. Now (IV.) will also undergo the same process, and the final

activated form will be (V.). But this is simply the activated form of (VI.). So that if there be, in the system an activating source, the structure (I.) and a hydrogen acceptor the affinity of which for hydrogen is greater than that of (V.), a reaction will take place to produce (VI.). This scheme, $I. \longrightarrow VI. + 2H$, represents in the clearest way Wieland's theory of the activation of hydrogen.

For the mechanism of oxidation of dibasic acids, substituted acids and other compounds, the original paper should be consulted.

A further point requires consideration here. Hydrogen acceptors fall into two classes : (i.) those that require activation by the cell (or the oxidising power of which is increased by the cell); and (ii.) those hydrogen acceptors the oxidising power of which is independent of the cell. To the former belong nitrates, formates and chlorates, and in the latter methylene blue.

There are three ways in which oxygen may be activated : (a) by iron (Warburg), (b) sulphydryl group (Hopkins), and (c) substances capable of peroxide formation, *i.e.*, those substances containing the group : $-CH = CN-$, $-CH = O$, and $-CH = N$. These become activated to $-\ddot{C}-CH_2$, etc. It seems possible, therefore, that oxygen may form a temporary link with the activated structure at the point of the unsaturated carbon atom :—



This compound having high oxidising power, and being, in effect, "active" oxygen, the very process which activates the hydrogen of the donator may also result in activating the oxygen. From the views put forward in this paper it will be seen that it is totally unnecessary to postulate the existence of numerous specific enzymes dealing with oxidations and reductions, but that it is possible to regard specificity of behaviour as belonging rather to the molecules themselves than to the enzymes.

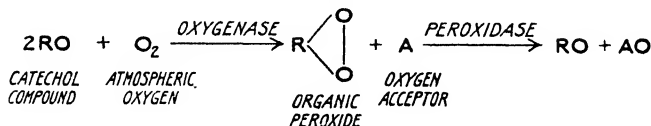
Enzymes concerned in Oxidation

A number of enzymes are known in plant cells which are directly concerned with oxidation processes. The most important

of these are : oxidase, peroxidase, zymase, carboxylase, tyrosinase and catalase.

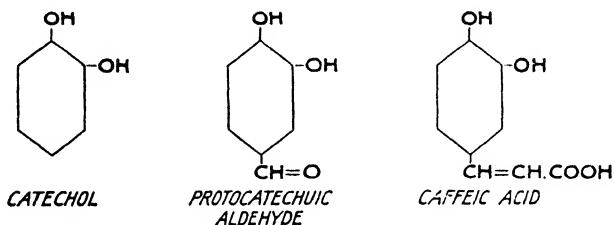
Oxidase and Peroxidase.—Oxidase is capable of carrying out a number of oxidations in the presence of free oxygen. Freshly prepared 1 per cent. alcoholic solution of tincture of guiacum is a delicate test for the presence of oxidase, when a deep blue colour is developed. If tincture of guiacum and hydrogen peroxide are brought into contact with certain tissues, such as horse-radish, a blue colour is formed, but no colour is given without the addition of hydrogen peroxide as well. This result is due to the presence of a peroxidase. It is only in the presence of a peroxide that a peroxidase is capable of carrying out its oxidative reactions, releasing oxygen from the peroxide. Thus guiaconic acid, which is found in tincture of guiacum and gives the blue oxidation product mentioned above with active oxygen, is stable to hydrogen peroxide alone, but on the addition of the peroxidase it is oxidised to the blue substance. According to the view originally propounded by Bach and Chodat, the oxidase system which acts directly on tincture of guiacum is composed of two enzymes : peroxidase and oxygenase, and the function of the latter is to activate the oxygen of the air to form the necessary peroxide.

Wheldale Onslow (1919, 1920) has found that various tissues which give the direct oxidase reaction with tincture of guiacum contain catechol derivatives. When solutions of these dihydroxyphenols are exposed to air, they slowly autoxidise, giving rise to brown oxidation products. These brownish products are accompanied by the formation of a peroxide, either an organic peroxide or hydrogen peroxide itself. In plants this reaction is catalysed by oxygenase. Thus, according to Onslow, the complete oxidase system consists of oxygenase, peroxidase and catechol compound, and their interaction may be graphically represented as follows :—



If the catechol compound be extracted with alcohol, the formation

of the peroxide of the system is prevented and the peroxidase is left in the tissue as a residue. On the addition of a little catechol, however, the system is once more reformed. Tissues which do not brown on exposure to air are said not to contain the catechol component. The most important of these catechol derivatives are catechol itself, protocatechuic aldehyde and caffeic acid. Derivatives of these substances are widely distributed in the plant world :—



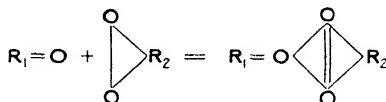
These results of Onslow have been criticised by Gallagher (1923), who considered that the peroxide present in plant tissues arises from some autoxidisable substance. One such substance that was isolated from plant tissues by this investigator was supposed to bear some resemblance to the lipins. Thus oxygenase, according to Gallagher, is an autoxidisable lecithin-like substance. Onslow's results, such as the blackening of tissues on injury, are said to be due, not to the presence of a catechol derivative, but to the action of tyrosinase on tyrosine to give the black pigment melanin. The positive reactions obtained by Onslow for catechol derivatives with ferric chloride are ascribed to the presence of tannins.

Gallagher (1924) visualised oxidation in the living cell by supposing that the lipin-like oxygenase is autoxidised to a super-

oxide of the type $R \begin{array}{c} \diagup O \\ \diagdown O \end{array}$. This superoxide is considered to be

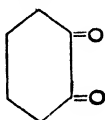
organic in nature, and to be derived from an aldehyde. It can fully recover its powers, temporarily lost by boiling, when allowed to stand after cooling. Iron was found to be present in the author's preparations of peroxidase, and since iron salts are known to activate the peroxidase activities of aqueous solutions

of aliphatic aldehydes—which already exhibit peroxidase functions when alone—he deduced that an aldehyde is the precursor or zymogen of the peroxidase, which is oxidised under the catalytic influence of iron to a substance of the type $R = O$. The mode of oxidation of such a system is supposed to consist in the combination of the two oxides with the production of a compound with an oxidation potential comparable to ozone, and higher than that of either of the components of the reaction ; this new substance is the actual oxidising agent :—

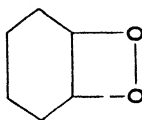


The number of postulations and assumptions made in this paper is too large to allow of any great credence being placed on the results. Robinson (1924) repeated Onslow's work, and has confirmed it in most particulars, and has also found that the oxidase of the basidiomycetes differs fundamentally from that of the higher plants and is composed of an enzyme-like peroxide and peroxidase. The lecithin-like substance described by Gallagher was isolated, but only gave the peroxide reaction after nineteen days with horse-radish and tincture of guaiacum. This is certainly too long a period to allow of any connection of this substance with the active oxidations of the living cell.

Recently Szent-Györgyi (1925) has made the suggestion that there is no need to postulate the existence of a peroxidase in the direct oxidase system. In his opinion, the only necessities for the direct acting oxidase system, which blues guaiacum resin without the presence of hydrogen peroxide, is an oxidase and a substrate containing a catechol derivative. By the action of the oxidase the catechol compound is converted into an *o*-quinone. These *o*-quinones give a blue colour with guaiacum tincture without the intervention of an enzyme. There are two possible types of *o*-quinones :—

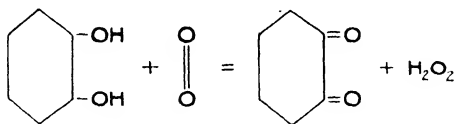


I. STABLE



II. UNSTABLE

Szent-Györgyi considered that peroxidases are merely attenuated forms of oxidase and are not specific enzymes. The presence of fully authenticated peroxidases in certain tissues is due, in his opinion, to the absence of catechol compounds; these substances being replaced by hydrogen peroxide or to a hydroquinone substrate which is oxidised to a *p*-quinone. These results have in general been confirmed by Onslow and Robinson (1926), who put forward the view that molecular oxygen acts as hydrogen acceptor in the reaction, oxidising the phenolic compound to a quinone, and at the same time forming hydrogen peroxide:—



They have been able to detect hydrogen peroxide qualitatively among the reaction products. In this particular case, the so-called oxygenase must play the *rôle* of a dehydrogenase. They were quite unable to bring about the oxidation of catechol anaerobically in the presence of methylene blue, so that there is no evidence that oxygenase can act in this capacity.

The nature of the peroxidase in horse-radish has been investigated by Willstätter and Stoll (1918) and Willstätter and Pollinger (1923). They were able to obtain a very active preparation by leaving the roots in running water to remove dialysable impurities. The roots were then treated with oxalic acid which precipitated the enzyme upon protein. The peroxidase and protein were then separated by treatment with alkali and successive adsorption on kaolin, which removed carbohydrate impurities as well as a glucoside. Following this treatment, the preparation was adsorbed on alumina, precipitated with tannin, reabsorbed on alumina and precipitated with alcohol. The view originally expressed by Willstätter was that the enzyme was of the nature of a nitrogenous glucoside, containing over 30 per cent. of pentose and an equimolecular proportion of glucose and iron. The first experiments with this preparation appeared to show a correlation of activity with the iron content, but later experiments failed to

show any such connection. In still more purified samples, neither protein nor carbohydrate was discovered, and the iron percentage was reduced to 0.06 per cent. It would thus seem that the iron is an impurity. On the other hand, the new and purer preparations still contained from 9.37 to 13.57 per cent. of nitrogen.

In connection with peroxidase and its reactions, Keilin (1925, 1926, 1929) has described a thermostable respiratory pigment to which he has given the name *cytochrome*. Cytochrome is widely distributed in the animal world and has also been shown to be present in plants. It occurs in bakers', but not brewers' yeast, and also in bacteria. With tincture of guaiacum and hydrogen peroxide, it gives the peroxidase reaction.

The oxidation and reduction of the pigment can be easily seen in yeast. In the reduced form it shows a clear absorption spectrum with four bands: *a*-6046, *b*-5665, *c*-5502, *d*-5210 Å. In the oxidised form of the pigment there are no clear absorption bands, but only a faint shading from 520–540/550–570. If a shallow tube (30 mm. high) be half-filled with bakers' yeast in water (20 per cent.) and the suspension examined with a Zeiss microspectroscope, the four absorption bands can be easily seen, but when air is rapidly bubbled through the suspension, the cytochrome becomes oxidised and the bands disappear. If the current of air be stopped, the pigment becomes reduced, and the four bands gradually reappear.

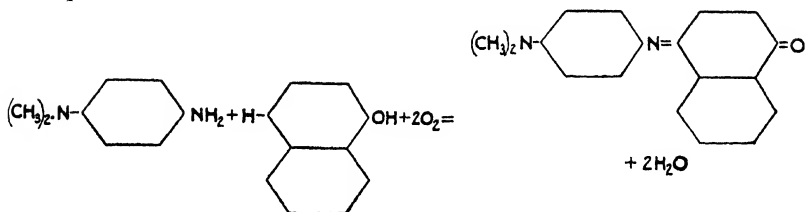
Potassium cyanide (N/10,000) has a narcotic effect and the cytochrome remains reduced. If a drop of potassium cyanide be added to a yeast suspension, kept at low temperature, and previously oxidised by a current of air, the cytochrome becomes immediately reduced, as though the cyanide were acting as a powerful reducing agent. Sodium pyrophosphate has an effect similar to the cyanide. On the other hand, substances such as formaldehyde, acetone and ethylurethane, which kill yeast, do not inhibit oxidation, but stop reduction and the cytochrome remains permanently oxidised.

In reality the yeast cells contain four hæmatin compounds: an unbound protohæmatin and three hæmatin compounds, *a'*, *b'*, *c'* of cytochrome, and these are capable of being oxidised and

reduced independently of each other. The three cytochromes, a' , b' and c' , are considered to be formed from the free intracellular protohæmatin which is present in all aerobic cells.

Keilin considered that hæmatin compounds are responsible for the peroxidase reactions of bacteria. Thus the aerobic bacteria (*Bacillus subtilis*, *B. proteus*, *B. megatherium* and others) contain a thermostable peroxidase and are rich in cytochrome, whereas the anaerobic bacteria (*B. sporogenes*, *Streptococcus acidilactici*), which do not give a peroxidase reaction, are completely devoid of hæmatin compounds. Of the four hæmatin compounds, the compounds a' , c' of cytochrome are not autoxidisable, while b' and the unbound protohæmatin are autoxidisable, and the latter in the reduced state will combine with carbon monoxide.

In addition to their thermostable peroxidase system, Keilin, contrary to the results of Harden and Zilva (1914), has also been able to show that yeast cells contain a powerful thermolabile oxidase system, which rapidly oxidises *p*-phenylenediamine to a dark purple quinonoid compound, and also reacts with the Nadi reagent, *p*-aminodimethylaniline and α -naphthol giving indo-phenol blue : —

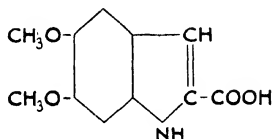


This oxidase is irreversibly destroyed at 70° C., and is markedly inhibited in its action by potassium cyanide and hydrogen sulphide, though sodium pyrophosphate has no effect upon its activity. All the factors which inhibit the activity of this oxidase or destroy it completely affect in the same way the oxygen uptake of the cells, which demonstrates that this "indophenol oxidase" plays an important part in cellular respiration.

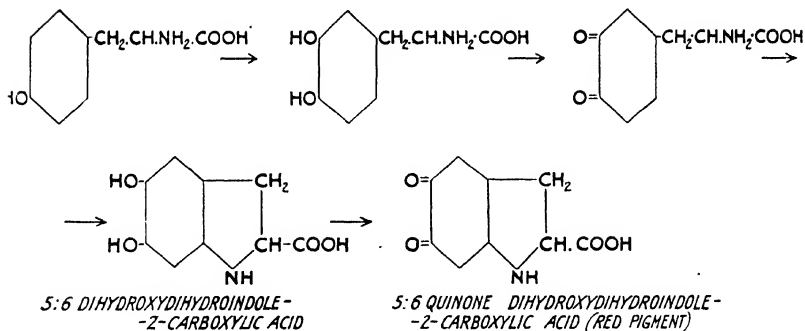
This oxidase is responsible for the oxidation of cytochrome, especially of the portions a' and c' which are non-autoxidisable, since the oxidation of cytochrome is abolished, or at any rate

change to give the colourless substance, and this, under the influence of the oxygen of the air, was oxidised to melanin.

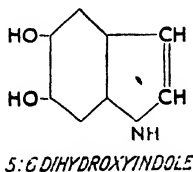
The matter proved to be more complex than the view put forward above. Raper (1927) has completely reinvestigated the problem, and he found that if the red compound were allowed to decolorise *in vacuo* or in the presence of sulphurous acid and then concentrated in an atmosphere of carbon dioxide, it methylated with methyl sulphate to give two crystalline products, one an acid and the other a feeble base. These were found to be indole derivatives. The acid had the structure of a 5:6-dimethoxy-indole-2-carboxylic acid :—



The changes to the red pigment are therefore represented as follows :—



The red pigment suffers slow auto-reduction with loss of carbon dioxide to give the colourless body which has the structure :—



and this, in the presence of atmospheric oxygen, is oxidised to melanin.

Carboxylase.—The enzyme carboxylase removes carbon dioxide from the carboxyl group of ketonic acids giving the corresponding aldehyde. Its function in alcoholic fermentation has already been considered and the matter will not be discussed further yet.

Catalase.—This enzyme is widely distributed in the plant kingdom and decomposes hydrogen peroxide into water and molecular oxygen. Its function is obscure, but it would appear in many cases to play a protective rôle. Loew has shown that in the oxidation of the purine bases by the xanthine oxidase, the enzyme is destroyed by the gradual accumulation of hydrogen peroxide. If, however, catalase be present, the oxidase remains unharmed, as the hydrogen peroxide formed in the reaction is quickly decomposed to water and oxygen. Loew therefore considered that catalase plays a protective function in living organisms.

The exact function of catalase in germination has been much investigated in recent years, but the problem still remains to be solved. According to Gračanin (1926), the activity of the catalase of germinating seeds generally increases to a maximum in about four or five days. After the fourth day there is a decrease in catalase content of the cotyledons, but an increase of the enzyme in the root to a constant value. In the fully developed plant the catalase is chiefly found in the leaves and roots. He also found that the catalase is chiefly located in the embryos of dicotyledonous seedlings, whereas in monocotyledons, such as maize, it is present in both embryo and endosperm and in small amount in the testa. From its presence in these structures, it presumably exercises some function in germination.

Morinaga (1925) found that the catalase content of dry rice is only one-tenth that of barley, oats, wheat or rye. Rice normally germinates under conditions in which free oxygen is not abundant. On the other hand, if rice be allowed to germinate under aerobic conditions, the catalase content rises to the same value as in barley, oats, wheat, and rye. Under anaerobic conditions, the catalase content of rice does not increase, but in a medium of low

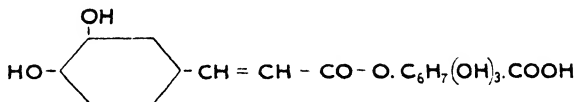
oxygen content there is a slow increase during germination. Hence the ratio of catalase increase is a function of the free oxygen of the medium.

Burge and Burge (1924) found that a fall in temperature produced a decrease in the catalase content of *Spirogyra* and a rise in temperature, an increase of catalase. This is in keeping with the fact that a fall in temperature decreases the catabolic activity of the plant, and that a rise increases it. Light was also found to increase the catalase content of *Spirogyra*, but its action was less effective than temperature.

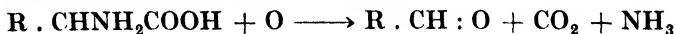
The reaction of the medium has an important influence on the activity of catalase. It is quickly destroyed in acid media.

The Oxidation of Fats and Proteins in the Plant

Little is known about the oxidation of fats and proteins in the plant cell. Oparin (1921, 1927) has investigated a depside, chlorogenic acid which is very widely distributed in the plant world, although strangely enough it does not occur among lichens, which form the chief source of most other depsides. This chlorogenic acid is optically active, and is not precipitated by gelatin and gives a green colour with ferric chloride. On hydrolysis with mineral acids it gives caffeic acid and quinic acid, and its constitution is accordingly written :—



According to Oparin, chlorogenic acid is easily oxidised by atmospheric oxygen with loss of four atoms of hydrogen giving a green pigment. The latter is capable of acting as a hydrogen acceptor, and therefore can play the part of an oxidising agent. The calcium salt of the fully oxidised acid has the formula: $\text{C}_{32}\text{H}_{32}\text{O}_{19}\text{Ca} \cdot 2\text{H}_2\text{O}$ and the reduced salt of the acid, the formula, $\text{C}_{32}\text{H}_{36}\text{O}_{19}\text{Ca} \cdot 2\text{H}_2\text{O}$. This chlorogenic acid is an active oxidising agent for α -amino-acids, peptides and peptones giving rise to carbon dioxide, ammonia and aldehyde.



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CHAPTER VI

THE PHYSIOLOGY OF GROWTH

Germination—Dormancy—Mycorrhiza—Growth—Elements needed to Build up Plant Tissues—Secondary Elements in Plant Nutrition—Auximones—Temperature—Light, including Etiolation—Photoperiodism—Electricity—Climatic Factors—The Frost Resistance of Plants—Carbohydrate/Nitrogen Ratio—Reproduction, including Heterothallism—The Nature of Growth Curves.

Germination

THE first inception of the living plant is from seed or spore. Subsequent developments based on the physiological processes of photosynthesis and respiration give rise to the mature individual. The birth of the new plant begins with the germination of the seed or spore. The seed of the higher plants is a highly specialised structure consisting in the majority of cases of a resting embryo with first leaves or cotyledons, plumule and radicle and supplied with reserve food material upon which it draws in the preliminary stages of germination. The spore of the lower plants, on the other hand, is no such specialised structure as the seed, and is incapable of withstanding prolonged periods of rest before the advent of suitable conditions for germination.¹ The seed with its resting embryo can survive far greater environmental adversities than the less protected spore and is capable of germination on the arrival of suitable conditions.

The necessary factors for the germination of seeds can be classified under four main heads :—

1. Water.
2. Oxygen.
3. Temperature.
4. Light.

¹ According to Ramsbottom, a case is recorded among the fungi where spores at least fifty years old were found to germinate.

Water is an absolute necessity for successful germination. The quantity of water taken up by different seeds is variable ; wheat varies between 40 to 60 per cent., peas from 84 to 106 per cent., and in other plants such as maize, the value lies between 35 and 40 per cent. of the dry weight. The first stages of water absorption are due to imbibition. The cells of the embryo and endosperm are non-vacuolate, and it is only in the later stages of the process that cell vacuoles are formed and osmotic pressure comes into play.

It is still a matter of considerable controversy whether the soaking of seeds in water improves their germinating power. Morinaga (1926) has studied the capacity which seeds have of germinating under water in seventy-eight different genera, and has found that forty-three of these genera germinated under water. Seeds of two of the genera germinated better under water than on moist filter paper, while in eighteen of the cases germination under water was equal to that on damp filter paper. On the whole, it was discovered that small seeds germinated better under water than on filter paper. If the oxygen-content of the water were increased, several seeds, normally incapable of germination under water, germinated readily.

Temperature also plays a fundamental part in germination. If the temperature be too low, germination fails to occur, if too high, the seed may be permanently injured and killed. Temperature affects the rate of entry of water into the seed as well as the growth rate of radicle and plumule, it also affects the resistance of the seed coat to the extrusion of the radicle. The temperatures at which different seeds germinate are different. *Phaseolus vulgaris* will show evidence of germination at 9° C., and the growth rate increases with temperature to 36° C. and ceases when 46° C. is reached. Rye will germinate at as low a temperature as 1° C., the maximum being 36° C. and the optimum 25° C. In all cases we are dealing with a minimum, maximum and optimum degree of temperature in germination ; although, as F. F. Blackman has shown, the " optimum " points are largely fictitious, varying with the time during which the factor has operated. The time-factor is of importance here, for the temperature may be too

high for further growth to take place, once the radicle has made its appearance ; in other words, for a brief period of time the high temperature has caused rapid growth, but the rate falls away owing to the operation of the time-factor.

Morinaga (1926) submitted the seeds of a number of genera to alternation of high and low temperature. As a general rule, each exposure to high temperature was for six hours, followed by exposure to a lower temperature for eighteen hours. Thus *Cynodon dactylon* germinated most readily with six-hour periods at 38° C., alternated with eighteen hour periods at 10° C., while alternation between 32° C. and 10° C. and 38° C. and 15° C. were less effective.

If oxygen be excluded, germination is inhibited, while certain seeds fail to germinate in the dark, and light is a requisite factor. According to Morinaga (1926) the seeds of *Typha latifolia* germinate more readily when the oxygen pressure of the air is reduced by partial replacement by hydrogen or nitrogen. The action of oxygen and light brings this discussion to the large and important question of the *dormancy of seeds*.

Dormancy.—It has already been stated that the seed of the higher plant is composed of an embryo which is differentiated into stem or plumule, radicle and cotyledons. The seed, as such, contains a minimum quantity of water, shows an absence of cell vacuoles ; and therefore in this condition chemical reactions, especially respiration, are reduced to a minimum. In such a state, the seed can retain its vitality over a prolonged period of time ; as much as fifty or eighty years in some exceptional cases. The seeds with the greatest vitality occur among the Leguminosæ. As a general rule, seeds which show a high vitality possess a tough testa which prevents the absorption of water and is impervious to the action of gases.

The whole question of the factors affecting dormancy of seeds has been reviewed at length by Crocker (1916), and an excellent summary of the physiology of dormancy is given in his paper. In general terms, Crocker found that dormancy or delayed germination can be attributed to one or more of the following causes :—

(a) Incomplete development of the embryo.

(b) Impermeability of the testa to water.

(c) Mechanical restraint offered to the expansion of the embryo and other seed contents by the seed coats.

(d) Inhibition and retardation of gases to, or, from the embryo, resulting in an accumulation of carbon dioxide within the tissues of the embryo, or in an insufficient supply of oxygen for germination.

(e) The necessity for the embryo itself, to undergo certain after-ripening processes before germination and growth under ordinary conditions becomes possible.

(f) Introduction by various means of a condition of dormancy in seeds previously capable of immediate germination.

To (f) Crocker applied the special name "secondary dormancy."

According to Rose (1919), freshly harvested seeds of *Tilia americana* with 10 per cent. or less of water-content, or seeds kept in warm storage for several months, fail to germinate when placed on a moist substratum at ordinary room temperatures. By numerous experiments he was able to show that the germination of the seeds could be brought about by a period of after-ripening in moist storage at 0° C. to 2° C., followed by a period of two or three weeks at 10° C. to 12° C. until germination was well started. To obtain vigorous growth of the seedlings a still higher temperature was needed. During the after-ripening process, it was discovered that the hydrogen-ion concentration of the seeds increased as well as the catalase and oxidase activity.

Rose also found that the freshly harvested seeds of *Sambucus canadensis* failed to germinate when sown at room temperature, but if they were kept in moist soil out of doors for the length of the winter, a high percentage germinated. He was unable to discover the factors influencing and controlling germination here. In *Rubus Idæus* he was able to show that dormancy was due to the high breaking strength of the endocarp. The presence or absence of light did not influence the germination of the seeds.

Pack (1921) ascertained that the seeds of *Juniperus* germinated readily if they were first maintained at a constant temperature of

5° C. for 100 days, to allow of after-ripening of the embryo. If the seeds, after the completion of the ripening process, were removed to a higher temperature (in this case 15°) they were thrown into a state of secondary dormancy. The following changes accompanied the after-ripening process: (1) an increase in the pH of the embryo; (2) increase of titratable acid; (3) decrease in stored fats and proteins; (4) increase in sugar-content; (5) translocation of fats from endosperm to embryo; (6) slight increase in the rate of respiration of the embryo; (7) increase in the value of the respiratory coefficient; (8) marked increase in catalase activity.

Kidd and West (1917) have shown that the seeds of *Brassica alba* sown in the presence of carbon dioxide could in certain circumstances be completely inhibited from germinating, and that this inhibition of germination might be maintained over prolonged periods; as much as twelve months in their experiments, during the whole of which time the seeds lay on a moist bed of sand. When the seeds were exposed to air, Kidd and West discovered that this condition of secondary dormancy could in most cases be terminated by drying the seeds or removing the testas without drying. No evidence of any changes in the seed coat could be found during this period of primary inhibition. On the other hand, it was shown that if the testas were removed with extreme care, the naked embryo remained dormant, and it was therefore suggested that a more stable condition of the tissues of the embryo had become established during this period of primary inhibition. Under the influence of carbon dioxide, embryos in this stable condition did not respond to normal environmental factors which govern the course of germination of ordinary embryos. Some very definite stimulus, either chemical or mechanical, was necessary to initiate cell division, and therefore the growth of the dormant embryo.

The condition of dormancy in the marine pea, *Lathyrus maritimus*, has been investigated by Stiles and Dellow (1924). The seed of this plant possesses a particularly tough testa which will not absorb water, even after immersion for a year, nor will the seed germinate when planted out. If, however, the testa be

broken, germination proceeds normally. Stiles and Dellow suggested that in its normal surroundings, *L. maritimus* propagates itself either by vegetable suckers or that the seeds are cast on the shingle and the testas cracked by the action of the waves on the pebbles.

It is a well-known fact that willow (*Salix*) seeds cannot retain their vitality for more than a few days. Yozo Nakajima (1926) has shown that the seeds of *Salix pierotii* and *S. japonica* lose their vitality under normal conditions in seven days, but if they are maintained at 0° C. the power of germination is retained for a longer period. The seeds show still greater vitality if they are kept at 0° C. under rather dry conditions. In such circumstances germination with *S. pierotii* will occur 360 days after harvesting the seeds.

Davis (1927) found that the seeds of *Cornus florida* were dormant when the fruit has first matured, and that this dormancy could not be broken by treatment with acids, ether or ethylene. A period of after-ripening of 100 to 130 days was necessary at temperatures varying between 0° C. to 10° C. before germination would take place. During the after-ripening there was an increase in starch, sugar and amino-acid content of the seeds, but little change in fats, phosphatides or acidity. The catalase activity of the seeds was closely parallel to the after-ripening process. The seeds of *Sambucus canadensis* and *Berberis thunbergii* required alternating temperatures for germination.

Since in the seeds of *Cornus florida* increased capacity for germination was accompanied by increased catalase activity as well as an increase in the amount of starch, sugar and soluble protein, while the respiration fell until germination actually began, Davis suggested that the process of after-ripening was closely linked with respiration. Further evidence for this view was given by the fact that if the seeds were exposed to higher temperatures (15° C. and over) there was an increase in readily usable food material, the catalase activity fell and the respiration rose.

Light.—The action of light on germination has been much to the fore in recent years, and most of the work on this particular aspect of germination has been conducted in Germany. It has

been known for a considerable time that the seeds of some plants do not germinate in the presence of light, while others refuse to germinate in the dark.

Busse (1925) found that the seeds of tobacco germinated more readily in the light than in darkness. Darkness, however, did not inhibit germination completely, but merely retarded it. Kinzel (1917), working with the seeds of *Aquilegia atrata*, kept them on moist blotting paper for ten years (1907-17). During the first year only 10 per cent. of the seeds germinated; the remaining 90 per cent. lay dormant in a perfectly healthy condition for the whole period of ten years until exposed to light, when 60 per cent. germinated in fifty days. Lehmann (1918) showed that the fully imbibed seeds of *Lythrum salicaria*, of which only a fraction germinated in the dark, gave a germination of about 50 per cent. in twenty-four hours after a brief exposure (1/10 second) to a light intensity of 730 candle-power at a distance of 1 metre. Lehmann supposed that light has some kind of catalytic effect on seeds that will only germinate in the light, converting the insoluble reserve foods into soluble substances. In the case of "dark" seeds, *i.e.*, those seeds that will only germinate in the dark, the light was supposed to activate fluorescent organic compounds which have a destructive effect upon the proteolytic enzymes present.

Further work on *Lythrum salicaria* has been carried out by Lakshmana Rao (1925), who allowed the seeds to germinate under constant conditions of temperature and varying light intensity. At higher temperatures (31° C. to 35° C.) with a single period of continuous lighting the product law was found to apply with very fair accuracy, that is to say, the percentage of germination was proportional to the intensity of the light and the time. At lower temperatures (25° C. to 28° C.) the action of the light was not so effective, and here germination was inversely proportional to the intensity of the light. These variations from the product law are explained on the results of experiments with intermittent light. In this case the effect was proportional to the sum of the exposures used. With long periods of darkness, the effect of light exposure increased in proportion to the quantity of light used up to a maximum value, while with still longer dark periods, the period

of light became less effective again, until finally the effect was less than with continuous light. Rao concluded from the results of his experiments that light had a direct effect on the embryo and not on the dead tissues of the testa.

Many organic and inorganic substances have the power of completely or partially counteracting the retarding influence of light on light-sensitive seeds. Kuhn (1916), working with different seeds, studied the inter-relation of various mineral acids and light and darkness as factors influencing germination. Seeds of *Phacelia tanacetifolia* on filter paper moistened with distilled water gave a germination of over 80 per cent. in the dark, but only 18 per cent. when exposed to light. By the substitution of weak solutions (0.1 M.) of different acids, Kuhn was able to raise the germination to 60 to 80 per cent. of that in the light. In the dark, however, the acids appeared to be without effect upon germination. Similar results were obtained with *Solanum lycopersicum* and *Amaranthus atropurpureus*.

Magnus (1920) failed to find this encouraging action of dilute acids on the germination of *P. tanacetifolia*. A so-called "false germination" takes place by the extrusion of the embryo. He also found that an aqueous extract of the seeds tended to inhibit germination in the light, but, curiously enough, not in the dark. Further, if a portion of the seed near the micropyle were removed, good germination occurred in the light. It is thus evident that some substance is secreted near this portion of the seed that is responsible for the failure to germinate in the light.

The action of light on seeds is a complex one. Lehmann (1919) found that the seeds of *Veronica tournefortii*, which normally germinate in the dark, at a temperature of 30° C., did not germinate in light or darkness, while solutions of potassium nitrate stimulated germination in either light or darkness; the results, taken as a whole, were better in the dark. Treatment with potassium nitrate in the presence of light resulted in an 80 to 90 per cent. increase in germination as compared with the controls in distilled water. It was also found that the higher the intensity of the light, the greater the retarding effect upon germination, and potassium nitrate was quite incapable of counteracting this

retarding influence. Thus with a light intensity of 440 candle-power at a distance of 1 metre and a temperature of 30° C., potassium nitrate had no effect upon germination.

According to Niethammer (1927), with increasing age the sensitivity of the seed to light decreases. It would appear that the place in which the seed ripens plays no part in its sensitivity to light, and he concluded that under natural conditions sensitivity of seeds to light is an important factor in germination.

It has usually been supposed that the seeds of *Viscum album* (Mistletoe) require a prolonged resting period of five months before they are capable of germination. Heinricher (1916) was able by suitable experimental conditions of high relative humidity and light intensity of 1,600 candle-power to force the germination of the seeds at any time during the winter.

Boerher (1916), in the course of an ecological survey of various forests, has come to the conclusion that light plays a distinct part in the problem of seed germination under natural conditions. In his opinion it has an indirect effect upon soil moisture, evaporation, and other factors.

The question of dormancy in relation to age, vitality and respiration of seeds and their oxidase and catalase content has been investigated by Crocker and Harrison (1918). They were able to show that when the freshly harvested seeds of Johnson grass received a preliminary incubation at 20° C. in a germinator they remained dormant for a year or more. If a portion of the grain surrounding the embryo were removed, prompt germination occurred. It is evident then that dormancy here was brought about by the structures surrounding the embryo. The catalase activity showed a marked reduction while the seeds were in the incubator at 20° C., a reduction of as much as 50 per cent. taking place in the first month. This fall in catalase content was accompanied by a fall in respiration; whereas there is normally a rise in such seeds kept under conditions of dry-storage. If this gradual fall in respiration be of general occurrence in fully imbibed but dormant seeds, and, further, if the assumption be made that the death of such seeds is dependent upon the utilization and destruction of the reserve foods present by respiratory activity,

this result must have an important bearing on the longevity of seeds under natural conditions. Brenchley (1918) found that many seeds would survive burial in the soil for as long as fifty-eight years.

Mycorrhiza.—Although in the large majority of cases temperature, oxygen, water-content and light or darkness are the principal factors concerned in germination, in a number of families of flowering plants germination is found to be dependent upon a fifth factor: the presence of a fungus. This state of affairs is especially prominent in the Orchidaceæ and Ericaceæ.

Unless the seeds of an orchid come into contact with its suitable fungus, no or very little germination occurs. It was on this account, although the scientific reason for the procedure was not understood, that orchid growers always sowed the seeds in soil in which orchids had already been growing. This association of fungus with higher plant, which is of wide occurrence in the vegetable world, was termed “mycorrhiza” by Frank. The mycorrhizal association of orchids has been widely studied, and now finds commercial application in horticulture. An excellent review of the whole of this subject has recently been published by Rayner (1927), and only certain aspects will be considered here.

The classical work of the Frenchman, Bernard, has largely cleared away the difficulties surrounding the germination of orchid seeds. The seeds of orchids are minute dust-like particles, produced in enormous quantities from a single capsule, and each seed possesses an undifferentiated embryo. The seeds prove to be quite sterile when sown in the ordinary way, but if the necessary fungus, here a species of *Rhizoctonia*, be present, germination proceeds rapidly and smoothly. In the case of *Bletilla hyacinthia*, Bernard found that in the absence of its fungus, germination was able to proceed to the first leaf stage, but this, as Ramsbottom (1922) pointed out, was probably a “last despairing effort” on the part of the seedling to survive in the absence of its fungus by the formation of its photosynthetic mechanism. In after-life the fungus is kept within the outer cells of the cortex of the root and never enters the endodermis or vascular cylinder.

An investigation of the rôle played by the fungus in the germination of orchid seeds has been carried out by Knudson (1922), who has shown that the germination of the seeds of a hybrid orchid, a cross between a *Laelia* and *Cattleya*, took place in the presence of sucrose solution and absence of fungus.

In the Ericaceæ the problem of the relation between plant and fungus has been exhaustively studied by Rayner (1915, 1916, 1922). Here, unlike the Orchidaceæ, the fungal mycelium extends through the whole plant and infection of the seeds is brought about in the ovary. By sterilising seeds of *Calluna vulgaris* in weak mercuric chloride solution, Rayner showed that the root system failed to develop in the young seedling, and that such leaves as were formed soon showed marked chlorosis. Infected seeds, on the other hand, developed an extensive root system and grew normally. This work of Rayner has been called in question by Christoph (1921), who considered that the presence of the fungus was not necessary for the complete development of *Calluna*, and that growth proceeded as readily in sterilised as in unsterilised cultures. Rayner has, however, confirmed her previous observations. The fungus concerned here is a *Phoma*, a member of the fungi imperfecti. According to Rayner (1929), *Vaccinium* presents a unique case among the Ericaceæ and exhibits the most highly evolved symbiotic relation discovered in this family. The fungus, as in *Calluna*, is distributed throughout the plant and extends to the ovaries and other organs of the flower. Rayner found it impossible to obtain seeds of *Vaccinium* free from fungus infection by sterilisation or removal of the testas previous to germination. All the tissues of the emerging seedling were subject to invasion by fungal mycelium, and it was probable that the normal germination of the seeds was bound up with this invasion.

The physiological relationship of fungus and higher plant is still a matter of controversy. It is probable that the mycorrhizal habit originated in a parasitic attack of the fungus upon the higher plant, and that the attack was at first repelled and later turned to advantage by controlling measures, with the final result that a fine balance of adjustment has been attained between

the two organisms; the fungus relieving the higher plant of certain of its metabolic functions.

Knudson (1925) held that the presence of the fungus facilitated the solution of insoluble carbohydrates until the assimilation mechanism had been firmly established. He found that *Rhizoctonia repens* induced germination of *Cattleya* seeds on a starch-containing medium and accelerated the germination of orchid seeds in a nutrient solution of sucrose. The fungus was able to effect germination *without infecting* the seed, due to its ability of digesting starch in the medium and modifying the pH to a value favourable for growth. On a peat and sphagnum substrate of nutrient solutions adjusted to a pH of 4.6 germination was as rapid as when the fungus was present; the especial factor for germination being that carbohydrate should be present.

In certain cases Rayner found that the fungus in *Calluna* could play the part of a parasite, especially if the seedling should happen to be at all weakly. The higher plant must therefore maintain a strict control over its fungal partner to prevent the latter from obtaining the upper hand and destroying the metabolic equilibrium.

The metabolic relations in after-life of fungus and higher plant is also obscure. The obvious statement to make in the circumstances is that the fungus must assimilate free nitrogen (see Goddard, 1913; Rayner, 1922; Senn, 1928). Experiments, however, have only to be made on this subject to be immediately contradicted. More recently, Fulmer and Christensen (1925) have grown yeast on a synthetic medium of phosphates and molasses, and found that this plant was able to assimilate free nitrogen at 30° C.; the assimilation was said to be a function of the pH of the medium. No gain was registered for some six to eight weeks, and it was on this account that Fulmer and Christensen considered that many of the older workers failed to obtain positive results of nitrogen fixation with fungi. Nielson Jones and Llewellyn Smith (1928) have brought forward considerable evidence to show that *Phoma radidis*, the endophyte of *Calluna vulgaris*, does possess the power of fixing molecular nitrogen. By means of an apparatus entirely closed to the external air, errors

due to the presence of ammonia and other gaseous nitrogen compounds were excluded, and the gaseous nitrogen used for the experiments was obtained electrolytically. The amount of nitrogen fixed was of the order of 0.00095 gm. for 0.345 gm. of glucose used ; a value very considerably less than for the nitrogen fixing bacteria. In the absence of either combined or atmospheric nitrogen, the nitrogen value was 0.00016 gm. for 0.21 gm. of glucose used.

Other workers have assumed that the fungus in some way assists in the absorption of salts and water from the soil. Exactly how this may occur is difficult to see. McLennen (1926), from her studies on the endophytic mycorrhiza of *Lolium subulatum*, *L. perenne*, *L. multiflorum* and *L. temulentum*, found that there was a metabolic exchange between fungus and higher plant, with the result that the higher plant obtained a supply of fat or oil, rather than a supply of nitrogenous food material. It is very probable that the metabolic functions of the fungus may be different in different families, and that in one case it may be concerned with the fixation of atmospheric nitrogen and in another with the production and exchange of fats.

Growth

Aided by assimilation and respiration, the seedling or sporeling is transformed into the mature individual. Growth is the final expression of successful metabolism. Without metabolism there can be no growth, and when metabolism ceases, death supervenes. It was early shown by Sachs that the only true criterion of growth is increase in dry-weight (material dried to constant weight at 100° C.), for transpiration and water absorption lead to considerable variations in fresh-weight, and no reliance can be placed on such figures. Estimation of dry-weight, however, has the disadvantage that the plant is killed, and now increase in leaf area is much used to measure growth, for it has been shown that increase in this organ is also an index of true growth (see below).

Both the anabolic and the catabolic sides of metabolism are necessary for growth. The living plant must be supplied with food, and it must also be supplied with energy, without which its

vital activities would soon cease. It is necessary, however, that the anabolic side of metabolism should be in excess of the catabolic, or no balance of material would be available for growth.

It has been shown by Boysen-Jensen (1918) that a balance is always available under ordinary conditions for growth. He attempted to elucidate the economic working of two biologically different types of plants: *Sinapis*, a sun-loving plant, and *Oxalis*, a shade type. In *Sinapis* the maximum intensity of assimilation was estimated at 6 mg. of carbon dioxide per 50 cm.² of leaf area every hour at 20° C. The dry-weight of a plant with a leaf area of 50 cm.² was 0.279 gm., and the respiratory rate was found to be 0.8 mg. of carbon dioxide for the same leaf area and at the same temperature. Boysen-Jensen was therefore able to calculate that the amount of dry-matter assimilated in the course of a day was 60 mg., and loss in dry-matter from respiration as 14 mg., or, in other words, the gain of material over loss was 46 mg., or 16.5 per cent. Here there is an ample margin of the anabolic over the catabolic side of metabolism to allow of fresh growth. In shade plants, such as *Oxalis*, the assimilation and respiration values are considerably lower than in *Sinapis*. In *Oxalis* itself the maximal assimilation was 0.8 mg. of carbon dioxide per 50 cm.² of leaf area every hour at 20° C., while the respiration rate varied between 0.1 to 0.2 mg. for a similar leaf area for the same time and temperature. Again, it is evident that there is a balance of material available for growth.

Growth Factors.—External and internal factors have a powerful influence on the growth of the living plant. If the factors are not suitable, growth may be retarded or even completely inhibited; or again, the absence of one or more factors may act in a detrimental manner on the well-being of the plant.

Among the principal factors concerned in growth are food and water. Without the right kind and quantity of food the plant cannot survive for any length of time. The whole subject of the food of plants is too large to be considered here in anything except brief outline. For a very full and detailed discussion of this subject, the treatise by Russell ("Soil Conditions and Plant Growth," 1927) should be consulted.

The Elements needed to Build Up Plant Tissues

Plant tissues are composed principally of the elements carbon, hydrogen, oxygen, nitrogen as well as sulphur, phosphorus, calcium magnesium and potassium. Besides these principal components, it is becoming increasingly apparent that particular elements are necessary in minute amounts to obtain normal growth in certain plants, and these appear to subserve the same purpose as the so-called vitamins in the animal economy. The qualitative study of the mineral nutrients necessary for plant growth is usually carried out in water culture solutions by leaving out one of the necessary elements. The method has the inherent weakness that the physiological balance of the solution is disturbed and the question of antagonism enters into the problem.

Carbon.—The only source of carbon for the higher green plants is the carbon dioxide of the air. This is present in the atmosphere in minute amount, approximately 3 parts in 10,000, but this low concentration is sufficient for the synthesis of carbohydrate. It has usually been considered that material increase of the carbon dioxide concentration has a toxic action on plants. This result, however, is due to the fact that the older workers employed highly impure sources of the gas, such as those from blast furnaces and gas flues. The carbon dioxide from such sources is always mixed with considerable amounts of sulphur dioxide and hydrogen sulphide, which are markedly toxic to plants. Bolas and Henderson (1928), using highly purified carbon dioxide and working under rigidly controlled conditions, found that artificial enrichment of the atmosphere with carbon dioxide resulted in a large increase in the dry-weight of cucumber as compared with the controls in normal air. The increase became evident at an early stage of the growth, usually two to three days from the beginning of the experiment. In one experiment, with a concentration of 31·3 parts of carbon dioxide per 10,000, the plants showed a percentage increase in dry-weight of $60\cdot6 \pm 8\cdot5$ over the controls with 3·9 parts of the gas in 10,000. These results will doubtless be of value in greenhouse horticulture in the future, and further observations should be of interest.

Certain of the lower green plants, especially the green algæ of the soil, can function as either autotrophic or heterotrophic organisms in nutrition. Bristol Roach (1926, 1927, 1928) found that the soil alga, *Scenedesmus costulatus* var. *chlorelloides*, could be grown in a liquid medium of nutrient mineral salts alone in the light or with the addition of 1 per cent. of different carbohydrates in the dark. With the addition of 1 per cent. glucose the organism was able to grow in the dark and retained its green colour. With maltose a similar result was obtained, but there appeared to be an initial "lag" period, preceding the straight line period of growth, while xylose was completely toxic to the organism. In the light, glucose and maltose gave the best growth, while the order of other carbohydrates was : galactose, sucrose and fructose. Other algæ which were found to be heterotrophic were : *Cystococcus* (sp.), *Chlorella* (sp.) and *Chlorococcum* (sp.), but they reacted very differently to the conditions imposed upon them. It is probable that it is not justifiable to regard the soil algæ as a homogeneous physiological unit in consideration of their relation to soil fertility. With *Scenedesmus costulatus* var. *chlorelloides* it was discovered that in high light intensity the organism was completely autotrophic in nutrition, but as the light intensity was reduced, glucose was taken up from the medium. It is possible that when this alga occurs in the lower layers of the soil, it grows at the expense of certain organic substances that are directly available. When, however, growing on the surface layers of soil, provided that the moisture conditions are suitable, photosynthesis takes place at a rate dependent on the intensity of the light.

Nitrogen.—The main source of nitrogen for the green plant is nitrates. Nitrogenous manures promote growth, their action is very swift, and they make the leaves a vivid green colour, causing rapid growth of leaf and stem. In large quantities they lead to rank and heavy growth and a tendency to susceptibility to infection from rust in wheat. Hursch has found that the amount of sclerenchyma is reduced in proportion to the collenchyma in wheat with heavy nitrogen manuring, thus favouring the attack of *Puccinia graminis*, the mycelium of which can only develop in the collenchyma. Gregory (1926) has shown that barley increases

its leaf area, but not its assimilation rate with addition of nitrate, whereas addition of phosphorus and potassium increase both assimilation rate and leaf area.

Appleton and Helms (1925) have ascertained that the rate of absorption of sodium nitrate by oats and cotton when applied at different stages of growth is more rapid the later the nitrate is applied. In both cases there is a close correlation between the rate of growth and the rate of nitrogen uptake. The effect of the amount of nitrogen supplied has been studied by Rippel and Ludwig (1926) on the growth of the sunflower; for smaller rates of nitrogen-supply the actual rate of growth is less, but in the first half of the growth curve the relative production of dry matter and the relative absorption of nitrogen are greater with the smaller dressings. Rippel and Ludwig (1925) have also attempted to correlate uptake of nitrogen with that of bases in the broad bean and oats grown in sand culture with or without the addition of combined nitrogen. The excess of nitrogen in the plants named above, which could have been absorbed in the form of nitrates combined with the bases present in the tissues (allowance being made for bases combined as sulphates and phosphates), is expressed as a percentage excess of nitrogen. As would be expected, this figure is much higher for broad beans when combined nitrogen is withheld. In the case of oats, the excess is still greater when nitrogen is given, whereas nitrogen-starved oats show a large excess of bases. Turner (1926) has demonstrated the contrast between the response of barley and maize compared with flax to variations in nitrogen supply. The former two crops show a large increase in the top to root ratio with increasing dressings of nitrate, which is due to a stimulation of the growth of the tops, and not to a depression of root formation. In the case of flax this effect is not very marked. Beyond a certain low level of nitrogenous dressing, further increases in nitrogen supply produce no appreciable change in the ratio of tops to roots.

Pearsall and Ewing (1929) have shown that plants grown in a medium containing large amounts of nitrates possess little protein, while low amounts of nitrogen lead to high protein-content. It is obvious, therefore, that the succulent condition of

the tissues of plants grown under high conditions of nitrogen manuring cannot be due to imbibition of water by protein. On the other hand, the ratio of protein to soluble nitrogen is always lower in plants receiving abundant nitrogen, *i.e.*, they have larger amounts of soluble nitrogen. The amino- and amido-nitrogen is always higher in high nitrogen manuring, and the chlorophyll content is also higher. The *pH* of the plant tissues under conditions of high nitrogen manuring is higher than with scanty nitrogen supply. This perhaps may be correlated with the fact that such plants as sorrel manufacture relatively larger quantities of organic acid, *e.g.*, oxalic acid, in soil deficient in nitrogen. The water-content of the tissues with high nitrogen is always considerably greater than with low nitrogen. Pearsall and Ewing suggested that since abundant nitrogen leads to an accumulation of amino-acids, these influence the metabolism in such a way that the production of organic acid is reduced, leading to an increase in the *pH* of the tissues (*i.e.*, alkalinity). This high *pH* and high amino-acid content leads to a greater swelling of the protoplasmic colloids, and it is perhaps due to this feature that there is higher water-content and reduced transpiration of plants growing under conditions of high nitrogen manuring.

Phosphorus.—The best source of phosphorus for plants is from phosphates. Phosphates promote root growth and have an important effect on the ripening of grain. The function of the phosphorus in the cell is difficult to ascertain. The presence of phosphorus appears necessary for mitotic division, possibly on account of the fact that phosphorus forms an integral part of the nucleus.

It is a well-known fact that the soil factors governing the supply of phosphate to the growing plant are considerably different from those concerned in the supply of most other nutrients. Von Wrangell (1926) distinguished three separate factors for the supply of phosphate from the soil : (1) the phosphate concentration of the soil solution ; (2) the rate at which this concentration is restored after disturbance of the equilibrium between soil and soil solution ; (3) the total reserve of soluble phosphate in the soil. The first factor is considered to be of especial importance,

because here one is dealing with easily soluble material as well as with substances of low solubility, the concentration of which largely depends on the presence or absence of other ions. Mac-Gillivray (1927) has found that in phosphorus-starved tomatoes there is a re-utilisation of the phosphorus present; about half of the total amount is found in the fruit, irrespective of treatment, although, if there be a shortage of phosphorus, the size and number of fruits is much decreased.

Brenchley (1929) has investigated the influence of phosphate on barley at different periods of growth. The plants were grown in water culture with full phosphate allowance and also with no phosphate. The plants grown in full phosphate solution were deprived of phosphate after varying initial periods and *vice versa*, i.e., plants grown in the absence of phosphate were supplied with the salt after initial periods of deprivation. Provision of phosphate for the first six weeks allowed normal growth to take place, shown by the number of ears, tillers and grain produced, as well as dry-weight. Shorter initial periods of phosphate allowance led to serious shortage in these respects. If the phosphate were withheld for four weeks, tiller growth was not affected, but no ears were produced. With still longer initial periods of deprivation, growth was steadily depressed in all respects, and the usual bushy growth tended to pass over to the thin, lanky type. The amount of phosphate absorbed by the plant increased more or less in direct proportion to the length of time phosphate was given at the beginning of growth, but sufficient was taken up in the first six weeks to allow of the plant making maximum dry-weight. The absence of phosphate in the early stages of growth, on the other hand, led to an extremely rapid drop in the ultimate amount of phosphate taken up by the plant.

It is evident that the importance of phosphate lies in the early stages of growth, and its application is of the most vital importance. Provided a sufficiency of phosphate be supplied in the early weeks of growth, tillering, ear, grain formation and increase in dry-weight proceed normally. These results are in contradiction to those of Pember (1917) and Pember and McLean (1918), who found that barley can utilise a deficiency of phosphate at any

time in the life of the plant. This, as Brenchley points out, may well be due to the fact that they used well water in their experiments, which contains traces of phosphate, and, further, that they did not allow the tillers but only the main shoot to develop, so that such phosphate as was present was concentrated in its sphere of action.

Gericke (1925), working with wheat, obtained the maximum dry-weight when the plants were grown in nutrient solutions for four weeks and then transferred to solutions containing no phosphate. Decrease occurred in the ultimate dry-weight as the initial period with phosphate was lengthened; the plants receiving phosphate throughout the experimental period being amongst those with the lowest dry-weight. Brenchley never found this sharp fall in barley, although certainly there was decrease in the dry-weight with continuous supply of phosphate.

It would seem that the phosphate requirements of cereals at different times of development are by no means fixed or definite, but are influenced by various factors, such as environmental conditions (seasons, spacing of plants, time of sowing, etc.). Nevertheless, it seems to be a definitely established fact that the most critical period for phosphate nutrition is in the early stages of growth.

Potassium.—Potassium aids in the production and translocation of carbohydrate, and appears to be of especial importance to leguminous plants. Maskell (1927), in a series of observations on the production of starch in the potato, using a special technique which allowed observations to be made in the field, found that in plants growing on plots which had received respectively potassium chloride, potassium sulphate, low-grade potash salts and no potassium, statistical examination of the data showed that starch production was appreciably increased by potassium sulphate, but not by fertilisers containing potassium chloride. The rate of translocation of starch from the leaflets on the potassium sulphate plots was also increased, but at the same time this varied significantly with other factors, of which, intensity of solar radiation and age were important.

According to Gregory and Richards (1929), barley (variety,
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Plumage Archer) grown in sand culture with full manuring, nitrogen deficient, phosphate deficient or potassium deficient gave the following results :—

| <i>Respiration.</i> | | <i>Assimilation.</i> | |
|----------------------------|--------------|--|---|
| | | (Low light intensity). | (High light intensity). |
| Fully manured. | Normal. | Unaffected by age of plant. | Falling with age. |
| N ₂ deficient. | Subnormal. | Normal. Unaffected by age. | Subnormal. Falling with age. |
| PO ₄ deficient. | Normal. | Slightly supernormal and falling with age. | Slightly supernormal. Falling with age. |
| K deficient. | Supernormal. | Subnormal. | Subnormal. |

They further found that the absence of potassium has less effect on tillering than absence of either nitrogen or phosphorus, and this tillering proceeds till death is about to take place. Rapid death of the tillers now occurs, and after this the new leaves which appear on the surviving tillers are darker green in colour, and from this time onwards the death of the leaves keeps pace with new development, so that at most two living leaves are found on each tiller. It was discovered that the death of the leaves sets free potassium, which is immediately translocated to freshly developing leaves.

Calcium.—The absence of calcium causes stunting and discolouring of roots, as well as brown spotting and subsequent death of leaves. The older physiologists associated calcium with the formation of proteins as well as with the formation of the material composing the cell wall. Recent work has tended to confirm this view. Parker and Truog (1920) explained the association between protein and calcium as being due to the precipitation of acids formed as by-products in protein synthesis by this element; for example, the Leguminosæ, which are rich in protein, are also rich in calcium. Newton (1923) did not support the conclusions of Parker and Truog. He found that the intake of calcium by peas and wheat in water culture solution is not proportional to the nitrogen content. Newton explained the high calcium content of leguminous plants when grown in the soil to be due to the much larger amounts of carbon dioxide evolved by the roots of such plants in comparison with non-leguminous plants, and that this results in an increased uptake of calcium which does not neces-

sarily bear any relation to the nitrogen content of the plants. The argument, however, is not entirely conclusive, and the subject requires further examination.

Neither barium nor strontium can replace calcium, as both these elements are extremely toxic to the well-being of the plant.

Secondary Elements in Plant Nutrition

It has become increasingly apparent in recent years that besides the elements enumerated above as being essential in plant nutrition, other elements in minute amount are also necessary for the normal growth of the plant. The early pioneering investigations of Mazé (1916) foreshadowed the possibility that, by more refined methods, the ten elements postulated by Knop and the older school of physiologists as satisfying all the requirements of plant growth might be insufficient. This view has been abundantly justified by a number of recent investigations.

The method of experimentation is to grow the plants in the usual nutrient solutions prepared from highly purified chemicals. In such circumstances many plants fail to grow normally. The addition of minute amounts of certain elements, *e.g.*, zinc, manganese, aluminium and boron, has, in a number of cases, brought about normal growth.

Iron.—Although iron does not form an integral part of the molecules of the chlorophyll pigments, nevertheless a small amount of iron is necessary for the production of these pigments in leaves. In 1920, Oddo and Polacci put forward the claim that iron could be replaced in culture solutions by the magnesium salt of pyrrole carboxylic acid. This statement, however, could not be substantiated by Deuber (1926), who found that in no case did the substitution of the magnesium salt of this acid prevent chlorosis in cowpea, soya-bean, corn and *Spirodela*. In fact, in concentrations of 0.001 to 0.250 gm., the salt was definitely toxic to the plants.

Boron.—It was shown by Warington (1923) that the broad bean, *Vicia faba*, only attained full development when grown in the presence of a trace of boron, and that no other element could

replace it. In the absence of boron the plants showed a stunted development, and death eventually occurred in a very characteristic manner by the blackening and withering of the tips (Fig. 42). The best results were obtained with amounts of the order of one part in a million, while larger quantities (1 in 5,000) were toxic. Warington came to the conclusion that boron is essential for other leguminous crops, but not for barley. Brenchley and Thornton



FIG. 42. Broad beans (*Vicia faba*) grown in the presence of various concentrations of boric acid. Left to right, 1 in 5,000; 1 in 50,000; 1 in 100,000; 1 in 500,000; control with no boric acid. (After Warington, *Anns. Bot.*)

(1925) have found that the nodules surrounding the roots of *Vicia faba*, which are responsible for nitrogen assimilation, are much reduced in the absence of boron. The vascular structure becomes defective, the strands being either entirely absent or only running for a short way into the nodule; there is at the same time a reduced development of bacteroid forms, and the amount of nitrogen assimilation is much below normal, and, in fact, the bacteria have been found to change their symbiotic habit for one of parasitism, attacking the protoplasm of the host (Fig. 43 & 44).

Warington (1926) has confirmed this work of Brenchley and Thornton, and found considerable hypertrophy of the cambial cells of *Vicia faba* in the absence of boron, as well as frequent disintegration of phloem and ground tissue and poor development of xylem.

Brenchley and Warington (1927) have carried the matter a

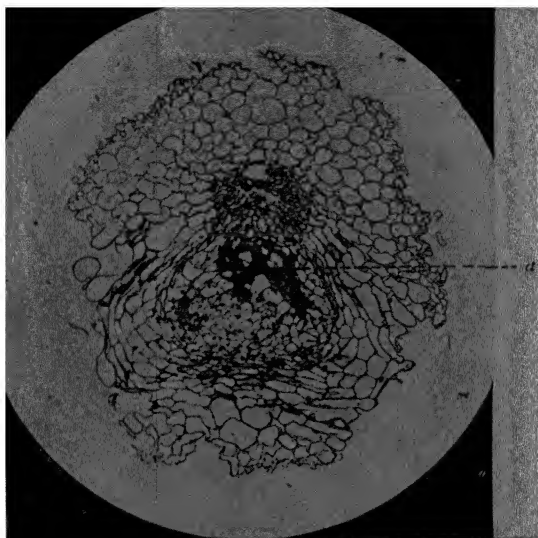


FIG. 43.—Nodule of *Vicia faba* without strands, in which bacteria are attacking the tissues. At (a) masses of bacteria have broken the cells of the host and turgid uninfected cells can be seen projecting into the disintegrating tissue. (After Brenchley and Thornton, *Proc. Roy. Soc. Lond.*)

stage further, and have discovered that the need of boron is independent of the pH of the medium in which the plants are growing. Even an insoluble borate, such as aluminium borate, is effective in this connection. Boron is essential for several leguminous plants as well as melon, whereas cereals and candytuft can develop in its absence. The boron plays an integral part in the calcium metabolism of the plant; in its absence *Vicia faba* is unable to assimilate calcium.

Collings (1927) stated that, contrary to Warington's results, boron is not essential for the complete development of the soy-bean, although in water culture it does have a markedly stimulating effect. Sommer and Lipman (1926) found that boron is essential for the development of corn, peas, sunflower, vetch, barley, buckwheat, dahlias, lettuce, potatoes, millet, castor beans, sugar beet, kafia, sorghum, flax, mustard and pumpkin. They found that

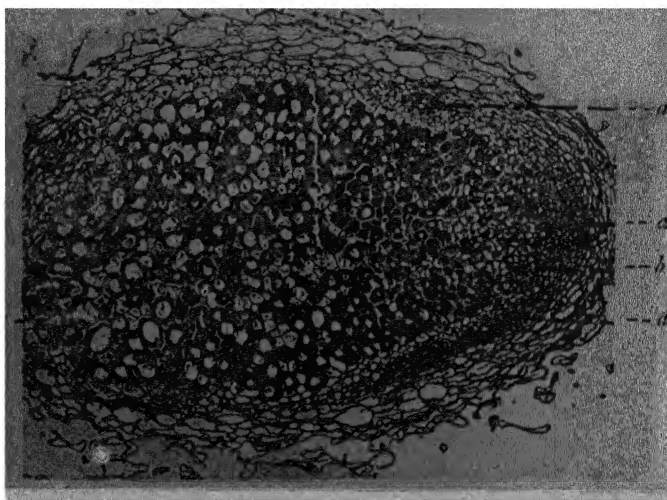


FIG. 44. Longitudinal section of large nodule of *Vicia faba* showing normal structure in the presence of boron : (a) meristem cap, (b) swollen vacuolated cells, (c) bacteroidal tissue, (d) vascular strands. (After Brechley and Thornton, *Proc. Roy. Soc. Lond.*)

dicotyledons respond more quickly to boron than do monocotyledons, and it may well be on this account that Warington failed to find any stimulating effect of boron on barley. The effect in certain cases may not become apparent for a week or two, or even for as long as a month (barley). They also found that zinc is necessary for wheat, buckwheat, broad beans and kidney beans.

Johnston and Dore (1929) found that boron is essential for the normal growth of the tomato. In the absence of this element the

plants show four distinct types of injury : (a) death of the terminal growing point of the stem ; (b) breaking down of the conducting tissues in the stem ; (c) characteristic brittleness of stem and petiole ; and (d) roots extremely poor in growth and of a brownish unhealthy colour. There appears to be evidence of a quantitative relationship between the amount of growth and the amount of boron present in the nutrient medium : the greatest amount of

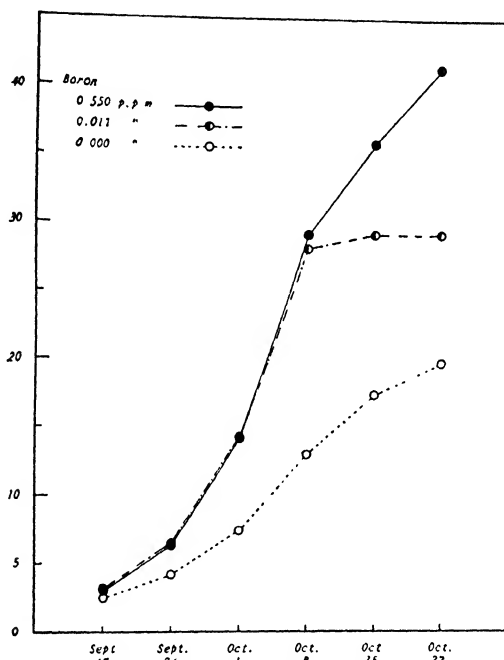


FIG. 45. —Average height (centimetres) of tomato plants grown in nutrient solutions deficient in boron and in solutions to which 0.011 and 0.55 parts per million boron had been added. (After Johnston and Dore, *Plant Physiol.*)

growth was obtained when the concentration of boron was 0.550 part per million (Fig. 45).

Manganese.—Minute amounts of manganese have also an important effect on the growth of certain plants. McHargue (1926) grew maize, onion, cucumber, lettuce, wheat, spinach, oats,

beans, tomato and peas in pot culture in quartz sand specially freed from manganese salts. Two series of experiments were set up: (1) in which plants were grown with the addition of manganese carbonate; and (2) in the absence of any manganese compounds. In the controls, when the manganese contents of the seeds, principally contained in the testas and embryo, were exhausted, growth ceased, and the leaves showed signs of chlorosis, and the starch and sugar content also fell. Normal growth was resumed when the manganese was added to the soil. Neither copper, iron, zinc nor arsenic was able to replace manganese in this respect, and McHargue considered it to be of equal importance to iron in the formation of the chlorophyll system.

Bertrand and Rosenblatt (1922) have found that leaves can be divided into four classes. In the first, which includes beet and *Aucuba*, the highest manganese content occurs in the youngest leaves. Later in the life of the plant the manganese content of the plant falls. In the second group, which includes box, ivy, iris and yew, the manganese content at first decreases and then rises to a higher value than before. In the third group, for example, *Syringa*, privet and chestnut, the manganese content increases at first very rapidly to a maximum, and this value then falls, while in the fourth and last group there is a steady increase in manganese content with age. Further investigations of Bertrand and Rosenblatt have shown that in *Nicotiana rustica* and *Lilium lancifolium*, the highest manganese content occurs in those organs which are most active biochemically. Thus, the flowers possess more manganese than the leaves and young leaves more than older leaves and green stems.

McLean (1927) claimed that manganese salts pumped into leaves *viâ* the stomata by means of a porometer are equally as effective as when they are added to the soil. Schreiner and Dawson (1927) have discovered that if tomatoes be grown in the field and also in pot culture, using a naturally calcareous soil practically free from manganese, the addition of 25.5 parts per million of manganese sulphate produces normal growth in contrast to chlorosis in the untreated soils.

Silicon.—The older physiologists considered silicon to be of

importance in the nutrition of the Gramineæ, and that its presence gave strength to the straw. Later experiments, however, have shown that silicates act by causing an increased assimilation of phosphate. Lemmermann and Wiessmann (1922) considered that perhaps silicates or colloidal silica could replace or partially replace phosphates. This view has since been shown to be untenable, and Lemmermann himself has abandoned it. Lemmermann, Wiessmann and Sammet (1925) have now published the results of a further investigation, and have shown quite definitely that the favourable effect of silica is to be correlated with an increased assimilation of phosphates. Němec (1927) has found that the amount of phosphoric acid taken up by the plant is proportional to the amount of water-soluble silicate in the soil.

Auximones.—Bottomley (1917, 1920) considered from a number of experiments on the aquatic ferns, *Salvinia* and *Azolla*, and the common duckweed, *Lemna minor*, that, in addition to the ordinary culture medium (Detmer and Knop) made up of mineral salts, there was needed another factor for successful growth, and to this he gave the name *auximone*. By employing aquatics, Bottomley did away with the artificial conditions of growing normal land plants in culture solution. He found it necessary to add organic matter to obtain healthy plants, and found that aqueous extracts of bacterised peat and material containing nucleic acids gave the best results. The amounts necessary were said to be minute, and these growth-promoting substances were considered to have a resemblance to the vitamins of the animal biochemist. Mockeridge (1920, 1924) claimed to have confirmed Bottomley's results, and Rosenheim (1917) alleged that they held good for the growth of *Primula malacoides*.

This view of Bottomley on the necessity for subsidiary growth factors or auximones has not met with universal acceptance. Clark and Roller (1924) have shown that certain lower plants can be grown for months on end in a solution of purely inorganic salts, provided that the physiological balance of the solutions be correct. Similarly, Saeger (1925) has been unable to confirm Bottomley's results, and found normal growth of *Spirodela*, *Lemna* and other aquatics in ordinary culture solutions in the absence of organic

matter, provided that the solutions were diluted ten times. Wolfe (1926) has confirmed the work of Clark and Roller and Saeger, and suggested that the word auximone should now be dropped from the literature. Mockeridge (1927), in a reply to these investigators, pointed out that their media may have become contaminated with bacteria which have a powerful effect on the growth of *Lemna*, and that it may be on this account they were able to grow these aquatics for extended periods in purely mineral culture solutions.

Ashby (1929B) has now shown that the addition of organic matter does have a definite influence on the growth of *Lemna*. As Ashby pointed out, Bottomley and his co-workers made no attempt to control external conditions, especially the pH of the medium. This latter factor is especially important, as *Lemna* is very sensitive to changes in pH. Ashby grew *Lemna* under rigidly controlled conditions in dilute culture solutions of mineral salts alone, and also with the addition of organic matter (aqueous extract of horse dung). It was found that the *Lemna* could grow in culture solutions of pure mineral salts indefinitely, but the addition of organic matter produced certain remarkable results. The cultures were grown under constant conditions of light and temperature, and in constantly circulating air. Statistically examined, the results showed that the plants growing in cultures with the addition of dung extract (0.2 part per million) showed an increase in frond area and cell size over the controls. The number of chloroplasts per frond also increased, resulting in an increased rate of photosynthesis and therefore in an increased growth-rate. The influence of the organic matter is apparently catalytic in nature, for increase in the amount of organic matter added in greater amount than 2.0 parts per million caused no increase in the growth-rate. It would therefore appear that though organic matter is not essential for the growth of *Lemna*, its addition does have the effect of increasing the growth-rate to a remarkable extent, thus confirming Bottomley's work.

Temperature

Since both the physiological processes of carbon assimilation and respiration are accelerated by a rise in temperature, and

again, since assimilation is always in excess of respiration under normal conditions of metabolism, it follows that an increase of temperature will lead to an increase of growth. The range of temperature suitable for growth is restricted. Too high a temperature leads to a collapse of the metabolic machine, and brings about the death of the plant, and, similarly, if the temperature be

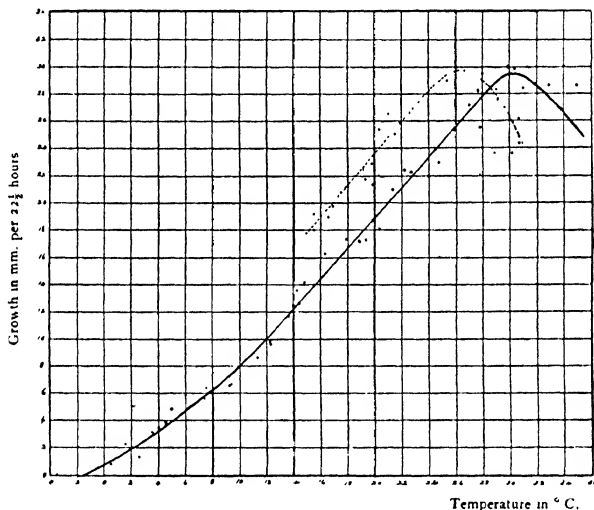


FIG. 46. - Curves illustrating the effect of temperature on the growth of roots of pea seedlings between 2° C. and 36° C. (After Leitch, *Ann. Bot.*)

too low, the metabolic rate is very considerably lowered, and growth is brought to a standstill.

According to Leitch (1916), the effect of temperature on the growth-rate of *Pisum sativum* is shown by a uniform curve for the range of temperature—2° C. to 29° C. (Fig. 46)—which closely resembles the curves obtained for respiration by Kuijper. Above 29° C., marked fluctuations make their appearance, and no single curve can express the relationship between growth-rate and temperature. Hence, for each rise in temperature a separate curve must be employed to express the rate of growth in successive periods of time. F. F. Blackman's "time-factor" is of importance

here. At 30° C. and 35° C. the rate of growth in the first ten minutes is the highest attained, while in the first half-hour there is a rapid fall, which in turn is succeeded by a second maximum showing recovery, and thereafter there is a gradual fall (Fig. 47). At 40° C. decrease in growth is rapid and uniform, and no recovery is registered. The coefficient for a rise in temperature of 10° shows a distinct falling off as the temperature increases, and from Leitch's results it is only between 10° C. and 20° C. that the

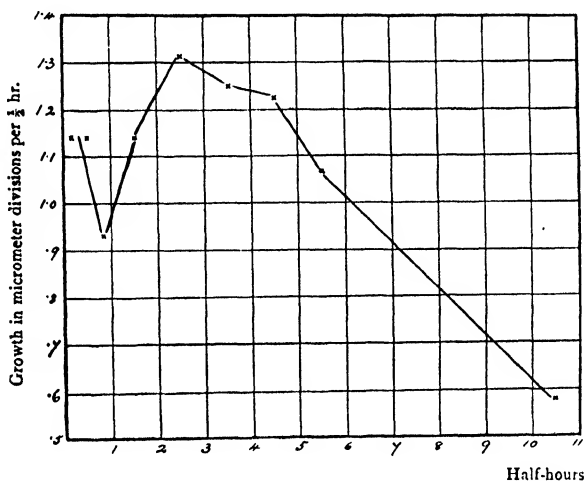


FIG. 47.—Graph of growth of pea seedling roots at 35° C. (After Leitch, *Ann. Bot.*)

coefficient value lies between 2 and 3, so that the complete curve is not a Van't Hoff curve. The greatest growth occurs at 30.3° C. Leitch distinguished four main points from her results: (1) the minimum temperature, (2) the maximum temperature, (3) the optimum temperature, and (4) the maximum rate temperature. The minimum temperature is the lowest temperature at which growth takes place, the maximum temperature is the highest temperature at which the process will occur, while the optimum temperature is the highest temperature at which no time-factor is involved, and the maximum rate temperature

is that temperature at which the process attains its highest intensity.

The variations discovered by Leitch at higher temperatures may well be due to a number of factors, the powers of activity of which have been increased at unequal rates. The solubility of different food materials, the stability of various enzymes that are present in the cells, and the accumulation of the end-products of respiration are all affected in different ways by an increase in temperature.

Priestley and Pearsall (1922), in a critical examination of Leitch's results, pointed out that the growth of radicles is dependent upon the chemical reactions involved in the cells of the growing-point. According to these investigators, increase in temperature will increase both growth and hydrolysis, but the tissues of the growing point quickly use up the food immediately available, with the result that a decrease in growth must obtain for that period of time required for the food materials in their journey down to the apical regions of the root. The arrival of this food is alleged to account for the second maximum in Leitch's curve. Finally, growth is diminished and brought to a standstill by the disorganisation of metabolism. This explanation certainly has the merit of ingenuity, but Leitch's results can be explained by any number of hypotheses should sufficient thought be given them.

Luyten, Joustra and Blaauw (1926) found that the earlier or later flowering of the Darwin tulip is dependent on the storage temperature in the previous summer. Bulbs stored at a temperature of 25° C. to 28° C. flower at the usual time; if, however, the bulbs have been stored at lower temperatures, the period of flowering under field conditions is accelerated by almost three weeks. Again, flower formation in the tulip is possible at a very much lower temperature than in the hyacinth.

Gregory (1921) has found that the rate of increase in area of cucumber leaves grown under continuous conditions of artificial light falls off with the first measurement of area. Gregory considered that the "detrimental factor" concerned here may well have been the high temperatures which had to be maintained

during the course of the experiments, which tended to increase the rate of respiration ; and he put forward three suggestions to account for the falling off of the growth rate :—

1. The growth ceases through the incipient starvation due to high respiration values, the concomitant of high temperature.

2. The detrimental effect of high temperatures and low light intensity is due to a change in the distribution of assimilated material in the plant.

3. To a direct action on leaf growth or to the factors acting simultaneously.

In a second series of experiments, Gregory (1928A) grew cucumber under conditions of constant light intensity (1,500 lux) and humidity at the following temperatures : 63° F., 70·8° F., 76·8° F., 84·4° F. and 90·3° F. Growth was measured as leaf area and dry-weight.

The growth of the total leaf area at higher temperatures conformed to the equation :—

$$\frac{1}{A} \cdot \frac{dA}{dt} = \frac{r}{t^n}$$

where A is the leaf area, t is the time that has elapsed from germination, and n a measure of the growth rate. The value of the exponent n increased with rising temperature : at 76·8° F., $n = 0.517$; at 84·4° F., $n = 0.613$; and at 95° F., $n = 1$. This indicates that with high temperatures, the falling off of relative leaf growth rate steadily increases.

The foliage leaves alone show growth curves which conform to a simple parabolic function :—

$$\log A = r \log t + \log A_0 ; \quad \frac{1}{A} \cdot \frac{dA}{dt} = \frac{r}{t}.$$

where A is the leaf area at a time t , A_0 is the leaf area at time unity, and r is the measure of the growth rate. At sub-optimal temperatures the value r remains constant, but falls rapidly at supra-optimal temperatures. The relative leaf growth rate is thus independent of temperature after the unfolding of the first foliage leaf.

According to Gregory, at sub-optimal temperatures relative leaf growth rate is independent of temperature, but is controlled by light intensity. Reduction of the light intensity or shortening the period of illumination leads to a smaller leaf area. The temperature affects leaf growth at sub-optimal temperatures by its action on the developmental rate in the apical bud, thus controlling the rate of unfolding of the first foliage leaf.

At supra-optimal temperatures a time-factor operates which tends to reduce relative leaf growth rate. This is due in part to a redistribution of material in the plant, more growth relatively by the stem, and, in part, to the direct effect of high temperature on the cell divisions in the leaf primordia, leading to a reduction in the number of cells produced before and after expansion.

At all temperatures employed in these experiments and with the light intensity used, there was a fall in the relative leaf growth rate with time. A second time-factor must therefore be considered as operating due to the low light intensity. If light alone be operating detrimentally, this time-factor should disappear with increase in light intensity, and this was found to be the case.

Light

In the vast majority of cases light is a large factor in the growth of plants. The effect of light on growth is fundamentally different from that of heat. A number of fungi, for example, can pass through the whole of their life-cycle in darkness, while in the greater number of land plants the root system is intolerant of light. In general terms, it can be said that light possesses a formative influence, and is also indirectly important, since it provides the necessary energy for photosynthesis.

Light has a strong formative influence on plant organs, such as stem and leaves, which are markedly altered morphologically by prolonged exposure to darkness. Apart from alterations in the colour of the green pigments of the stem and leaves, plants exhibit other characteristics when exposed to darkness; peculiarities which are summarised in the term *etiolation*. Etiolation phenomena are best studied in plants with abundant supplies of reserve

food, such as the dahlia or potato, since darkness deprives a plant of its assimilating powers. The general characteristics of etiolated plants are prolonged internodes and a suppression of the leaf lamina which becomes scale-like in shape. The xylem does not reach the same development as that of the normal plant grown in the light, and the etiolated shoot is soft, sappy and weak.

According to Priestley and Ewing (1923), the stems of *Vicia faba*, *Solanum tuberosum*, and *Pisum sativum* develop a true primary endodermis with Casparian strip when grown in complete darkness, in place of a starch sheath. This endodermis is considered to restrict the supply of nutrient sap necessary for the growth to tissues within the endodermal cylinder and the apex. The development of the endodermis in the dark is said to account for the excessive elongation of the stem, as only the cells at the base of the meristematic tissues which cap the end of the endodermal cylinder receive adequate supplies of food. Growth of this meristematic tissue adds to the length of the stem, and growth in length is favoured at the expense of the normal lateral growth of leaf and cortex; thus, for example, the angles of the stem of *Vicia faba* opposite the main vascular bundles fail to develop, and the stem remains rounded in cross-section. Occasionally it was found in *P. sativum* and *V. faba* that on prolonged exposure to darkness, the Casparian strip may fail to develop though growth continues, and in such circumstances there is a greater development of lateral leaf extensions. In *Phaseolus multiflorus* no endodermis develops in the dark, and therefore the stem remains rounded in outline. Priestley (1926) claimed that in *V. faba* a constant feature of the etiolated shoot is that the cortical bundles seem to divide relatively much earlier than in the non-etiolated plant. In the etiolated shoot the starch sheath is replaced by a primary endodermis. A striking stain which is alleged to show this endodermis is Nile Blue. From the reactions of the layer to osmic acid, the staining with Nile Blue seems to be due to the presence of fatty substances. The cells below the meristematic region are very fully packed with starch. Priestley concluded that the main morphological and structural features associated with etiolation are determined by a redistribution of meristematic

growth at the shoot apex, following on the greater difficulty experienced by the meristem in drawing nourishment from the vascular supply, because when grown in the dark, the walls between the vascular strands and meristem are rendered relatively impermeable by the retention within them of protein and fatty material that form the surface of the protoplast.

Unpublished observations by the present author have shown that *V. faba* does not develop a primary endodermis with Casparian strip when grown in complete darkness. In its early growth the apical meristem of the etiolated plant is certainly packed with starch which gradually disappears, until finally the starch granules in the starch sheath itself also disappear, but the walls never develop a Casparian dot. In any case, it is placing a heavy burden on a single layer of cells to claim that great morphological changes in the plant body are brought about by their presence, and it is difficult to conceive why the migration of fats should lead to these alterations in shape. The plant is a complex metabolic machine, and, as Gregory (1928) has pointed out, the action of light "is not inconsistent with a hypothesis of a master photochemical reaction independent of carbon assimilation leading to the formation of a substance directly involved in leaf expansion." There is a minimum light intensity below which growth cannot proceed, and unless this level of light intensity is maintained, a time-factor makes its appearance, leading to continuous reduction in relative leaf growth rate. This reduction of growth rate may well be due to a fall in the growth rate of the roots. In barley, certainly, root growth and leaf growth are closely correlated. This close correlation may be due to the fact that the leaf supplies carbohydrate which is important for the growth of the roots, and the roots supply nitrate which is necessary for the growth of the leaves. The action of hormones in plant physiology is only now beginning to be realised, and no doubt further investigations will lead to considerable extensions of our knowledge of plant metabolism in the future.

Short exposures to light have a marked effect on the development of the leaf lamina in etiolated plants. Trumpf (1924) exposed plants of *Phaseolus multiflorus* for varying periods of

time, such as thirty minutes, two hours, four hours and twelve hours, to artificial light, and found that even the shortest exposure to light produced profound morphological changes. The internodes became much shorter and the lamina broader. In a second series of experiments the plants were illuminated for one, five, ten and thirty minutes, and closely resembled plants exposed to a longer period in light, with the exception that plants having the longest daily exposure (ten to thirty minutes), although having well developed laminas, showed no development of chlorophyll.



FIG. 48.—Effect of brief light exposure on the broad bean (*Vicia faba*). Reading from left to right, 4 minutes daily ; 2 minutes daily ; 1 minute daily ; continuous darkness. (After Priestley, *New Phyt.*)

Thus, in changes of form, the quantity of light is the factor concerned, and its action is not produced indirectly on the assimilating system. Trumpf also ascertained that in light of different wave-length, the blue rays and, to a certain extent, the red rays favoured elongation of the stem, while the blue light markedly induced lamina formation. Priestley (1925) has confirmed these observations (Fig. 48), and also found that completely etiolated plants show no signs of lateral leaf development and always retain their plumular hook.

values lay rhythmically about a straight line. In general terms, the growth increased with increase of light intensity up to 700 foot-candles, but showed a considerable fall at 1,400 foot-candles. At the latter intensity the light had a definitely harmful effect upon the plants.

Ashby made the suggestion that after the optimum of light intensity is reached (the optimum being somewhere between 700 and 1,400 foot-candles), the reduction in the rate of growth at supra-optimal light intensities is probably due to a reduction in the chlorophyll content of the fronds. There is, however, some other detrimental factor concerned as well, since with reduction in the periods of light there is a reduction in the growth rate without any corresponding reduction in the amount of chlorophyll in the fronds.

The Effect of different Parts of the Spectrum on Growth.—The various parts of the spectrum have very different effects upon the growth and reproduction of plants. Many of the older experiments on this subject are vitiated by the fact that no account was taken of the energy relations involved. It is obviously necessary to have particular regard to the fact that, in experiments of this nature, due care is taken that the same energy relations prevail in all the experiments. If, for example, plants are being grown under blue and red screens to discover the action of blue and red rays on growth, the energy values of the two kinds of glass must be the same; should the blue glass absorb more light energy than the red, no comparison can be made between the effects of the blue and red rays on growth. .

Schanz (1919) grew plants under glass which transmitted definite parts of the spectrum. Eight beds were covered with different coloured glasses, and in the first five beds the light transmitted passed from violet to red, and in the final three beds combinations of glasses allowed yellow, green and blue-violet light to pass through. He found that taller plants were produced when the short rays of sunlight were removed. The maximum height was obtained under the red and the minimum under the blue-violet rays. This was found to be true for cucumber, *Fuchsia*, *Chrysanthemum*, *Lobelia*, *Begonia* and *Oxalis*, while

potatoes and beet showed the weakest growth in yellow light, stronger in green, and still larger and healthier in the blue-violet region. The development of chlorophyll in soy-beans, beans and potatoes was more rapid when the short rays were cut off and the most rapid development took place in the red rays. With lettuce, the chlorophyll was not produced in normal amount in yellow or green light, but developed fully in violet. It appeared from Schanz's experiments that ultra-violet light was necessary for the development of anthocyanin pigments, for even the flowers were pale in colour if the very short rays were removed. The time of flowering hastened in *Fuchsia*, tomatoes and beans, and the number of flowers and fruit produced was increased as the short wave-lengths of light were cut off. With red, yellow, green and blue-violet rays the number of flowers was reduced and the time of flowering postponed. The general opinion of this author is that the short wave-lengths of light are detrimental to growth; especially the rays in the ultra-violet region.

Popp (1926) has considerably extended these observations. A number of different species were grown in five specially constructed greenhouses, which transmitted the following parts of the spectrum :—

- House* (1).—From red end of spectrum to $312\mu\mu$.
,, (2).—All wave-lengths to $296\mu\mu$.
,, (3).—Ultra-violet rays eliminated.
,, (4).—All rays shorter than $472\mu\mu$ eliminated.
,, (5).—All rays shorter than $529\mu\mu$ eliminated.

A wide variety of plants was used in these experiments, which included, among others: *Nicotiana tabacum*, *Daucus carota*, *Petunia hybrida*, *Helianthus cucumerifolius*, *Soja max*, *Mirabilis jalapa*, *Coleus blumei*, *Fagopyrum vulgare*, *Lycopersicum esculentum*, *Holeus* (sp.) and *Sorghum sudanensis*.

The most striking results were obtained in houses (4) and (5), in which the entire blue-violet rays were eliminated. The plants showed decided signs of etiolation, although a good green colour, and in some cases (tomato) an even greener appearance was shown than by plants in the other houses. The stems were tall and

slender, the internodes greatly elongated, while soy-beans (*Soja max*) changed their habit of growth and became climbers. The leaves showed a tendency towards crinkling and rolling, which was pronounced in the case of *Helianthus cucumerifolius*, *Petunia hybrida*, *Nicotiana tabacum* and *Mirabilis jalapa*. There was considerable development of loose palisade tissue in the leaf, except in the soy-bean, which showed a considerable delay in reaching maturity, compared with the plants in the other houses.

The removal of the ultra-violet rays (house (3)) had little effect on the general growth and appearance of the plants. They were perhaps slightly taller and bloomed earlier than in houses (1) and (2). Plants in houses (1), (2) and (3) possessed a vigorous and healthy appearance. The rate of germination was unaffected in any of the houses. In the first two or three weeks stem elongation was greatest in houses (4) and (5), but later the rate fell below plants in the other houses. The removal of the blue-violet end of the spectrum uniformly resulted in decreased stem thickness; the smallest stems occurred in houses (1) and (2). The removal of the blue-violet rays also had a marked effect in some species on the flowering time; plants in houses (4) and (5) uniformly flowered later than in the other houses, except soy-beans, while sunflowers practically failed to flower and the number of flowers produced in the other species was greatly reduced. The dry-weight of the plants was lowest in houses (4) and (5), and the percentage of moisture greater. A chemical analysis of the plants showed that the greatest amount of nitrogen and the lowest percentage of total carbohydrate occurred in houses (4) and (5).

The results of these experiments indicate very clearly that blue-violet rays are necessary for good and healthy growth. The absence of rays shorter than $529\text{ }\mu\mu$ results in more or less etiolation. The intensity of the light is not an important factor in this connection, for normal growth is obtained when the plant receives the full spectrum of daylight at an intensity which at all times is less than that of the house in which all wave-lengths shorter than $427\text{ }\mu\mu$ are removed, and only slightly greater than that of the house in which wave-lengths shorter than $529\text{ }\mu\mu$ are eliminated.

Pfeiffer (1928) has found that plants receiving the full spectrum of sunlight make better growth, so far as differentiation of tissue is concerned, than plants that are only grown in the visible spectrum. In the latter case, the development of the vascular tissue is weak, the stems are spindle-like, the root development is poor, and the leaves less thick. When most of the ultra-violet rays are excluded, the plants show less stocky growth and contain more moisture, and the same result is noticeable if the red end of the spectrum be emphasised.

Electricity

The effect of electricity on the growth of plants has been investigated on and off for the last 180 years with remarkably poor results. The first pioneer in this branch of plant physiology appears to have been one Mr. Maimbray, of Edinburgh, who, according to Joseph Priestley in his "History and Present State of Electricity," published in 1776, electrified two myrtle trees "during the whole month of October, 1746, when they put forth small branches and blossomed sooner than other shrubs which had not been electrified." The Abbé Nollet, Court Physician to Louis XV., hearing of Maimbray's experiment, repeated it in France, and found that an electric discharge stimulated germination and growth of mustard.

Round about this time (1776) other views began to become prevalent. According to Koestlin and Ingen-Housz, electricity had a harmful effect on plant growth. In spite of the large amount of work that has been carried out on this subject with varying positive and negative results, none of the investigators concerned appears to have considered either the quantitative aspect of the matter or the plant. This quiet neglect of environmental and other factors concerned is not to be wondered at, since the workers in this field were either physicists, who were therefore unable to appreciate the biological side of the problem, or biologists whose knowledge of physics was of the scantiest nature.

Passing to more recent work, V. H. Blackman and Jørgensen

(1916, 1917), working with an overhead electric discharge, have shown a greater appreciation of the complexity of the subject and the difficulties involved in an investigation of this kind. They were able to ascertain that oats treated with an overhead discharge show a marked superiority over untreated plants. The electrified plants were larger and of a deeper green than the controls. The discharge in one set of experiments was continued for an average of five hours a day with the following results :—

| | Total grain. | Total straw. | Percentage increase of grain. | Percentage increase of straw. |
|---------------------|--------------|--------------|-------------------------------|-------------------------------|
| Electrified | 1309 | 2476 | 30 | 58 |
| Control | 1008 | 1572 | | |

The difficulty of confining the discharge to a special area was reduced by lowering the height of the wires. The amount of the discharge was 3 amperes at 50 volts.

In a second experiment two control plots were used, and the following values obtained :—

| | June 18. | June 25. | July 3, 1916. |
|-----------------------|----------|----------|---------------|
| Electrified | 19 | 24 | 32 inches. |
| Control I. | 14 | 19 | 21 „ |
| Control II. | 12 | 18 | 22 „ |

The electrified area gave a yield showing an increase of 20 bushels of grain over control I., an increase of 40 per cent., while the increase in straw was 89 per cent. A marked after-effect was noticed in the electrified plot, for there was a considerable increase in the crop sown in the following year on the same ground.

According to Blackman and Legg (1924), who worked on maize and barley in pot culture and used a current of strength 0.1×10^{-10} amperes, there was a percentage increase in the growth of the maize of 27 ± 5.8 and 18.0 ± 2.4 in barley. It was also dis-

covered that electrification of barley during the last month of the growing season was as effective as electrification during the whole season, and further that the discharge could be positive or negative in character. If currents of strengths 1×10^{-8} amperes, or higher, were used, the effect was found to be injurious.

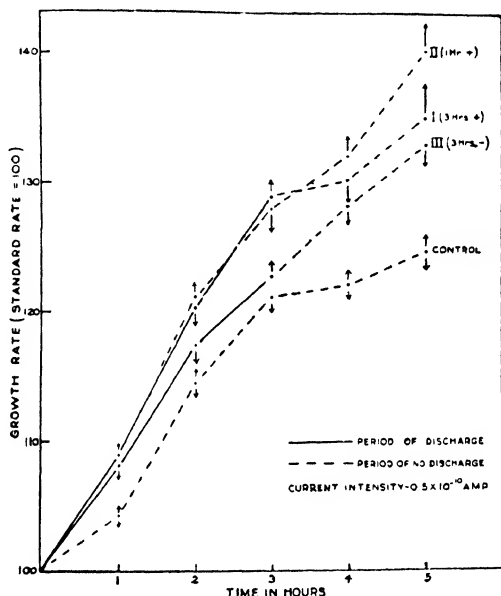


FIG. 50.—Graphs showing the mean hourly rates of growth of control coleoptiles and of coleoptiles exposed : (I.) to a *positive* discharge for the first three hours ; (II.) to a *positive* discharge for the first hour ; (III.) to a *negative* discharge for the first three hours. The growth rates are expressed as percentages of the “standard rate.” The length of the arrows indicates the size of probable error of the various means. (After V. H. Blackman, Legg and Gregory, *Proc. Roy. Soc. Lond.*)

The effect of a glow discharge on the growth of the coleoptile of *Hordeum vulgare* has been investigated by V. H. Blackman, Legg and Gregory (1923). A metallic point was charged to a high voltage and placed vertically over the coleoptile. The seedlings were grown in the dark in special culture tubes and a platinum wire was sealed into the glass so that the current actually passed

through the plant. The strength of the current used in the experiments was 0.5×10^{-10} amperes.

Since the individual coleoptiles showed a varying rate of growth, and as the normal rate of growth increased during the course of the experiment, the results were expressed as "relative hourly rates," the rate of growth of the coleoptile for the hour immediately preceding the experimental period of five hours was taken as the standard rate of growth for that coleoptile and expressed as 100. Plants were exposed (1) to a discharge of three hours with the point positively charged, (2) for one hour, and (3) treated to the discharge for three hours with the point negatively charged. It was found that during the discharge the current increased the rate of growth of the coleoptiles, and that the rate of growth was not only continued when the discharge was stopped, but grew even greater (Fig. 50). With the point negatively charged, this after-effect, although present, was not so great, and the rate of growth of the coleoptile tended to decrease during the discharge. This acceleration in the growth rate was entirely due to the current itself and not to the gaseous products of the discharge, for when a special platinum screen was inserted to cut off the current, no activation of growth was shown.

The growth of aberrant coleoptiles, however, mars the mean results obtained by these workers. In order to secure a uniform distribution of the effect of errors over the whole period of the experiment, Gregory and Batten (1925) held that in the estimation of the effect sought for, all measurements should have equal weight. They have achieved this by calculating from the hourly increments of growth, the "straight line of closest fit." A difficulty that was encountered in the course of the experiments was that the coleoptiles showed a well-marked rhythm in growth which had to be considered in making the calculations. The use of the formula :—

$$y - mx = c$$

where x is the number of hours since previous exposure to the current, c is the growth during that hour, and m is the average acceleration of growth in an hour, considerably simplifies the matter. From an examination of the data two important points

emerge : (a) that the slopes of the lines in corresponding series vary among themselves ; (b) even when the slopes are similar to the points of origin *c*, vary from experiment to experiment and are different in the control and electrified plants of a single series. By determining the correlation between the initial growth rate and the acceleration growth rate, a suitable correction may be made for a variation in subsequent growth rate due to the initial rate. Using such corrected data the growth rates of the controls and stimulated coleoptiles appear as lines of varying slope. It was found in all cases (where corrected data were used) that all the lines showed positive results and demonstrated the markedly stimulating effect of the discharge.

Collins, Flint and McLane (1929) have investigated the effect of an overhead discharge on the growth of barley and maize. They found that a current of intensity 10^{-9} amperes at night gave the most promising results with maize. The remainder of the experiments, however, did not give encouraging results, and in the majority of cases neither positive nor negative values for growth were obtained.

The precise reason why an electric current has a stimulating effect on plant growth is at present unknown. It was shown by Knight and Priestley (1914) that direct currents of 10^{-9} and 10^{-6} amperes do not increase or decrease the rate of respiration of germinating peas. An overhead discharge of 3×10^{-6} amperes also has no effect, while with higher currents there is an indefinite rise in the carbon dioxide output. This rise, however, was correlated with the rise in temperature due to the discharge. A further fact was discovered, that the gaseous products of the discharge have no effect on the germination of the peas, but are damaging to the seedlings. Further, the current apparently does not affect the photosynthetic rate. Marx (1929) has investigated the effect of electric currents on the assimilation rate of *Elodea canadensis* using Wilmot's bubble-counting technique. The products of electrolysis were not allowed to reach the plant and the strength of the current¹ was $1.5 \times 6 \times 10^{-7}$ milliamperes per

¹ The current density was calculated in terms of area of cross-section of the mass of water between the electrodes which was about 1720 cm.²

square centimetre. With such a current there was no increase or decrease in the assimilation rate. A current of 1 milliampere per square centimetre had a decidedly depressing effect. It is thus evident that the stimulating effect of electric discharge must be looked for elsewhere than on either photosynthesis or respiration.

Climatic Factors

The effect of such environmental factors as light, humidity and temperature on the growth of plants living under field conditions belongs more especially to a discussion on ecology, but certain limited aspects of the matter must be considered here. One or more of the above factors have already been considered in their individual action on the living plant; their mass effect has now to be discussed.

Hildebrandt (1917) has suggested that the leaflet measurements of the soy-bean might be used as a standard to measure the "climatic effectiveness" in the United States. McLean (1917) has employed the method to investigate the climatic action on the soy-bean in various parts of the U.S.A. The following measurements were made after two weeks and during the first month from sowing, at Oaklands (in the mountains of Maryland), and at Easton (on the eastern side of Chesapeake Bay); stem height, average number of leaves per plant, average length and width of mature leaves and the leaf-product, which was obtained by multiplying length by width of each leaf, the average leaf area and the dry-weight of the tops of the plants.

It was found that the rates of growth in terms of leaf surface and in terms of dry-weight varied in a similar manner with the same kind of variations in external conditions; whereas the growth rates measured in terms of stem elongation varied in another way with similar external conditions. The seasonal marches of the growth rates of the Oaklands plants were found to be considerably different from those prevailing at Easton. It would seem that temperature was clearly the limiting factor for growth during the first fortnight. During the second fourteen days, however, with similar external conditions prevailing, the

moisture relation (rainfall/evaporation ratio) appeared to be the limiting factor for growth, this being especially the case when the temperature was high. Thus it can come about with two plants in different phases of their growth and development, exposed to the same fluctuations of external conditions, that the limiting factor for one case may be very different to the limiting factor for the other. McLean pointed out that this may well be due to the difference between the internal conditions of the plant at different phases of its development.

Hildebrandt (1921) studied the effects of the climatic complexes on plant growth in nine different stations in Maryland for the summer of 1914. The effect of each complex was automatically integrated for soy-bean plants for a period of four weeks from sowing, new seeds being sown every two weeks. Measurements of stem elongation, leaf area, "leaf-product" and dry-weight were taken and the environmental factors studied were air-temperature, evaporating power of the air and the intensity and duration of sunlight. Indices of the total seasonal climatic efficiency derived by multiplying the seasonal average growth rate per day by the normal length in days of the growing season for the station in question were found to have the following values :—

| | |
|-----------------------|--------|
| Oaklands | 9,009 |
| Chewsville | 12,480 |
| College | 16,867 |
| Easton | 17,688 |
| Princess Anne | 19,005 |
| Coleman | 21,115 |
| Darlington | 23,688 |
| Baltimore | 25,422 |

The previous history of a plant has an important bearing on its subsequent behaviour. From a large number of experiments Balls (1918) concluded that the different behaviour of the plants, indicated by the crop of cotton, was the result of known environmental factors, provided always that true regard were paid to the distinction in time between the incidence of the conditioning factor and its manifestation in the crop. Thus the daily fluctua-

tions in the flowering curve were determined and controlled by weather conditions which were in force a month before the flowers opened. The importance of this principle of predetermination is very great, and has not sufficiently been realised in previous work.

Brenchley (1920) studied the effect of environmental factors on the growth rate of garden peas. The plants were grown in water culture and the rate of growth was expressed in terms of dry-weight. During the early seedling stage the rate of growth was associated with relatively warm days and nights, and bright sunshine had little significance. During the later periods of growth, increase was strongly associated with sunshine and warm days, but not significantly with night temperatures. These results were to be expected. In the early seedling stage the photosynthetic mechanism had not yet reached its full development, and therefore the young seedling could not make full use of the sunshine, while in the later stages of growth the perfection of the assimilation apparatus at once demonstrated the marked significance of sunshine.

Mason (1922) made a survey of the various external and internal factors affecting the shedding of the bolls and flower-heads of cotton in St. Vincent. He was able to show that the susceptibility to shedding became especially pronounced after growth-cessation in the main axis. This cessation of growth and increased susceptibility to shedding was found to be due to the deflection of the supply of elaborated food material from the apical portion of the plant to the fruit developing on the basal fruiting branches.

Overcast, humid days retarded the growth of the main axis; and low rates of evaporation, little direct solar radiation and periods of daytime rain were forerunners of increased rates of shedding. Mason attributed the retardation of the growth rate and increased rates of shedding to the check in the photosynthetic activity of the leaves, and drew the general conclusion that the proportion of shedding over any given period of time was the resultant of two opposing factors: (1) the rate at which food was synthesised by the plant; and (2) the rate at which it was utilised in the maturation of the fruit, and any check to (1) increased the rate of shedding.

Gregory (1926) has published a communication of considerable importance to the elucidation of climatic factors on the growth of barley. The barley was grown in pot culture and the water-content of the soil was maintained at 15 per cent. ; the optimum for the type of soil employed in the experiments. The seeds were graded and six sown in each pot ; after germination three of the seedlings were removed and the three most uniform seedlings left. Two types of manurial treatment were used : (a) 0.5 gm. sodium nitrate, 1 gm. superphosphate, 0.25 gm. potassium sulphate per plant ; and (b) 1 gm. sodium nitrate, 1 gm. potassium nitrate per plant. The assimilation rate in the two series proved to be the same. Three measurements of growth were employed. (1) net assimilation rate (dry-weight increase per unit leaf area per unit time) ; (2) relative growth rate of leaf surface (rate of growth per unit weight) ; and (3) relative rate of increase in dry-weight—the efficiency index (see p. 334). Measurements were also made of the environmental factors concerned : (a) maximum day temperature, (b) average day temperature, (c) minimum night temperature, (d) average night temperature, (e) total radiation in calories per square centimetre per week ; (f) hours bright sunshine, and (g) evaporating power of the air.

Dividing the growth cycle into two parts, Gregory found that the first part of the growth cycle was independent of the time and quantity of nitrogen added to the manure. There was a positive correlation of the assimilation rate with the day temperature and radiation, while there was no such correlation with the night temperature. In the absence of bright sunshine, in so far as the temperature did not fall, the high relative leaf growth rate led to a large leaf surface and so compensated for low net assimilation or *vice versâ*. This compensating effect, however, was partially masked by high nitrification rate in the soil which was associated with high soil temperature, and hence with total radiation, which in any case brought about an increase in leaf area. Secondly, relative leaf growth rate was largely independent of external factors, since the leaf is the metabolic factory of the plant and is independent, relatively speaking, of external factors.

Appleman and Eaton (1921) found that temperature was the

controlling factor of the rate of ripening of sweet corn under field conditions. For a wide range of temperature the rate of ripening strictly follows the Van't Hoff law.

The Frost Resistance of Plants

The influence of intense cold on plants is of economic importance in Europe and the American continent, and a number of investigations have been made on this subject to determine the precise action of extremely low temperatures on plant cells.

The effect of frost and low temperatures has been investigated by Harvey (1918, 1919). Using various succulent plants, Harvey found that one of the first effects of frost injury is the appearance of injected areas on the leaf due to the withdrawal of water from the cells and the subsequent displacement of air in the intercellular spaces by this water. In some plants the frozen cells may become stimulated into abnormal growth and tumour-like outgrowths are produced, very similar in appearance to those produced by parasitic fungi and bacteria. He compared the resistance to low temperatures and freezing of untreated and "hardened" plants of tomato and cabbage by exposure for a week or more to temperatures slightly above the freezing-point, and found in the cabbage that plants which had been kept for five days at 3° C. withstood exposure for half-an-hour to - 3° C., whereas the control plants were killed. Harvey considered that the chief effect of "hardening" is due to a change in the proteins of the protoplasm which prevents their precipitation as a result of the physical changes following on "hardening." Changes in the carbohydrate values are slight, whereas the amino-acid content increases. Any changes in the sugar content causing an increased depression of the freezing-point, or the nature of the epidermal cells, which may affect undercooling of the tissues by prevention of inoculation from ice formed on the surface, are relatively unimportant. Harvey (1922) has obtained very similar results for lettuce.

Tuttle (1919) and Lewis and Tuttle (1920), in an investigation of induced changes in the reserve material of evergreen herbaceous

plants, measured periodically from autumn to summer the osmotic pressures, electrical conductivities, proportion of electrolytes, as well as the amounts of glucose, maltose and sucrose in the leaves of *Picea excelsa*, *Linnaea borealis* and the cortical tissues of *Populus tremuloides*. No definite correlation could be found between these values and either daily or weekly variations of air-temperature. The sugars, on the other hand, showed a marked concentration during the winter and a progressive decrease from the maximum amount in the winter to the summer period.

A microscopic examination of the mesophyll structure of leaves of *Picea excelsa*, which had been subjected to a severe winter in North-West Canada, showed that the identity of the chloroplasts, which appeared bright green in colour, closely associated with the nucleus, was completely lost. During the autumn the whole of the starch content had disappeared and appeared to have been replaced by oil. Ice, according to these investigators, only makes its appearance in the living cells of *Pyrola* at temperatures below -31°C .

It must also be borne in mind in this connection that trees and shoots in cold climates become dormant at the close of the growing season without exposure to cold weather. Coville (1920) therefore considered that the generally accepted view, that the dormant conditions exhibited by plants in winter are due to low temperature is not necessarily correct. He brought forward the theory that the effect of low temperatures in dormant plants is closely connected with the transformation of starch into sugar; a change brought about by the weakening of the cell membranes, which become permeable to amylolytic enzymes, and these then act upon the starch grains stored in the cells. He also gave experimental data in support of this hypothesis, to show that growth may be stimulated by any process which will cause local injury to the tissues.

Johnston (1919, 1923) suggested that the ratio of water-content to dry-weight might serve as a possible index for the measurement of the winter hardiness of fruit buds. According to this same investigator (1922) there is a decrease in the hardiness of fruit buds with the approach of spring. Data are presented to show

that wet buds freeze at a considerably lower temperature than dry buds. It follows from this result that a cold spell following rain is especially dangerous to fruit trees. According to West and Edfelson (1921) the buds of peach, cherry and apricot, show different degrees of hardiness to frost at different stages in their development, and these differences are considered to be due to changes in the quality and quantity of the cell sap.

Newton (1922) in an investigation of the winter hardiness of wheat found that all the varieties used in his experiments increased in amino-nitrogen and water-soluble nitrogen during hardening. The hardest variety had the largest quantity of water-soluble nitrogen, but the relation was not constant throughout the series. The colloidal complex of fully hardened tissue was very resistant to freezing and could not be broken down by a freezing mixture with a theoretical temperature of -59.9°C . Newton (1924) also ascertained that the imbibition pressure of fresh leaves in the winter-hardened condition as determined by the pressure required to express the tissue fluids was in most cases directly related to hardiness. In one case an imbibition pressure of 600 atmospheres was recorded. With unhardened leaves no such relation could be discovered. The imbibition pressure of hardened leaves appeared to depend on the physical state of the cell colloids characteristic of living tissues, since this property was lost when the tissues were killed. It was found that the moisture-content of hardened tissues tended to be inversely proportional to hardiness. The hydrophylic colloids contained in the press juice were found to be directly proportional to hardiness. There appeared to be a certain amount of evidence that the ratio of amino-nitrogen to total nitrogen increased in all varieties in the late fall, indicating an association of protein-splitting with the later stages of the hardening process.

It was originally suggested in 1920 by MacDougal that pentosans were of the greatest importance in relation to the frost-resistance of plants. This view has been extended by Hooker (1920), who considered that the pentosans or rather some specific pentosan, functions in the plant tissue by holding water which is in the nature of adsorbed water or colloidal water, and that this type

actually does not freeze when the plant is subjected to ordinary winter conditions. The greater water-content found in tender tissue as compared with more hardy tissue is therefore due to an excess of free water. On the other hand, although hardy tissues contain less free water, they contain more adsorbed or colloidal water. It was found that the shoots of hardy varieties of apple, such as Wealthy and Yellow Transparent, always contained a higher pentosan content than the tenderer varieties, such as Missouri Pippin. In the majority of cases the base of the shoot contained more pentosan than the tip. Similar results have been obtained by Rosa (1920) for vegetables. Plants hardened by exposure to low temperatures or by withholding moisture showed a higher pentosan content than non-hardened plants, and the pentosan content increased with the hardening process. Rosa advanced a theory similar to that of Hooker to account for winter hardiness.

Newton and Brown (1926) could find no support for the suggestion that the pentosan content is correlated with hardiness in wheat. The bulk of the colloids in the cell contents consisted of proteins; 90 per cent. of the total protein of the plant being contained in the extracted fluids. The pentosans were almost entirely confined to the structural regions of the plant. Concentration of sugars in the plant increased most in the hardest varieties, thus giving them the greatest resistance to frost denaturation of proteins. It would therefore appear that the pentosans play little if any part in frost resistance. Similarly, Doyle and Clinch (1926) found that there is no apparent connection between cold resistance and pentosan content of the leaves of conifers.

The Carbohydrate/Nitrogen Ratio

Kraus and Kraybill (1918), in an elaborate biochemical investigation concerned with the nitrate and carbohydrate content of the tomato and the responses correlated with their presence, were able to recognise four main conditions :—

I. Though there be present an abundance of moisture and

mineral nutrients, including nitrates, yet without available carbohydrate supply, vegetative growth is weakened and the plants are non-fruitful.

II. An abundance of moisture and mineral nutrients, especially nitrates, coupled with available carbohydrate supply, makes for increased vegetation, barrenness and sterility.

III. A relative decrease of nitrates in proportion to the carbohydrate makes for an accumulation of the latter and also for fruitfulness, fertility and lessened vegetation.

IV. A further reduction of nitrates without inhibiting a possible increase of carbohydrates makes for a suppression both of vegetation and fruitfulness.

According to Kraus and Kraybill, "fruitfulness is associated neither with the highest nitrates nor with the highest carbohydrates, but with a condition of balance between them."

It was hoped that this work would give an impetus to the discovery of the underlying physiological reasons for such empirical horticultural practices as pruning and manuring. These investigators recognised the fact that fruit production is apparently a specialised vegetative function, usually more or less closely associated with gametic reproduction, and that the conditions for the initiation of flower primordia and even blooming are different for those accompanying fruit setting.

Since this original publication, a wealth of papers has been produced dealing with the carbohydrate/nitrogen ratio in different plants on the lines of Kraus and Kraybill and the subject has developed into quite a "vogue" among plant physiologists. Attempts have been made on all hands to explain nearly every manifestation of the plant with this ratio, although it is probable that Kraus and Kraybill themselves never meant to make the elaborate claims that later workers have superimposed on their paper.

Almost immediately following the work of Kraus and Kraybill, Woo (1919) published the results of his investigations on *Ameranthus retroflexus*. This plant has apparently an almost abnormal capacity for the absorption and retention of nitrates. Large quantities of nitrates may be present and yet the plant may not

be "forced out of reproduction," although the carbohydrate/nitrogen ratio is low. It would therefore appear from the results of this investigation that the ratio is different in different plants producing the same range of effects. This is an obvious conclusion, and Gurjur (1920) found that the ratio for tomato may vary between 2 and 19, but fruiting always occurred when the ratio was between 4 and 6.

The question of the application of the results of Kraus and Kraybill to the apple tree is beset with difficulties. In the apple, the fruiting spurs are all at the same stage of development at the same time, and it would thus appear that a more reliable indication of the relation between chemical composition and fruiting may be obtained if the investigations are confined to this part of the plant. Hooker (1920) has found that by correlating the carbohydrate and nitrogen content with the stage of development of the apple spur at the onset of the non-blooming year, there was low nitrogen and high carbohydrate content present, and that throughout the winter loss of both occurred up to the formation of the terminal buds. In the spring and summer, photosynthesis built up carbohydrate reserves, and the nitrogen still remained low. These conditions are considered to ensure fruit-bud formation. With the formation of the buds, the C/N ratio (carbohydrate/nitrogen ratio) changed rapidly within the spur, and the nitrogen accumulated to its highest concentration. Since the low C/N ratio brought about much vegetative growth during the summer and previous spring, the increased ratio now brought about flowering and fruit production. Hooker (1921) attempted to apply his results to orchards, by testing the effects of nitrogenous manuring at different periods of the year. The age of the tree is considered to be an important factor in this connection and the time of the year at which the manure is applied is also important.

Harvey and Murneek (1921) have shown that spurs defoliated in June have a lessened C/N ratio due to increased nitrogen and decreased carbohydrate, and as a result of this defoliation there was a decrease in flowering. Harvey (1923) has studied the rôle of carbohydrate and nitrogen upon the growth rate after defoliation and ringing at different periods of the year. He found that

defoliation retarded growth considerably, if it were effected during the period of maximum growth rate. On the other hand, defoliation earlier in the season showed a smaller retarding effect, though, if the defoliation were performed late in the season, there was very little retardation of growth, and it might even be accelerated. The result of ringing shoots was in general opposite to defoliation.

Roberts (1921) found evidence that apple trees may store a reserve of nitrogen in one season which may be utilised in a later season, when the external supply is low. He found that a high C/N ratio in the buds, *i.e.*, buds with a low nitrogen content, leads to little growth and the complete suppression of fruit-bud formation. However, a low ratio leads to much vegetative growth and very few fruit-buds. An intermediate value of the ratio leads to vigorous vegetation and abundant fruit-buds.

Gardner (1923) has attempted to apply the results obtained for apple spurs to strawberries. The yield of fruit is found to depend upon the nutritive conditions prevailing in the autumn at the time of formation of the fruit-buds. The maximum number of flower-head clusters is obtained when the conditions during the previous autumn are such as to provide a high carbohydrate content.

Hicks (1928) has considered the question of the C/N ratio in its relation to the growth of wheat. Only total carbon and total nitrogen were determined, and no attempt was made to distinguish between different carbohydrates. In the three strains of wheat used in these experiments: Marquis, Nevin Bearded and English Spring wheat, it was found that the embryos tend to reproduce similar C/N ratios, irrespective of the amounts of carbon and nitrogen in them. Early stages of germination were characterised by a low C/N ratio. Vegetative activity reduced the nitrogen percentage steadily and the carbon rose to a maximum at about half-way through the life-cycle. The percentage of carbon fell considerably before blooming, and, when a sufficiently high C/N ratio arose, flowering occurred.

This investigation is somewhat in the nature of the contemplation of the obvious, and the results could have been predicted

without the unnecessary toil of microanalysis which the author carried out with such laborious care. No possible significance can be placed on results which include the total carbon of the plant, because the continued production of dead tissue, such as xylem and sclerenchyma, will, of necessity, play an important rôle in the estimations and significance of the final results. More especially does this become apparent when the fact is considered that the nitrogen, in contrast to the carbon, is practically entirely used in active metabolism.

The significance of the C/N ratio in the interpretation of the results of photoperiodism has already been considered and will not be further discussed here.

The Physiology of Reproduction

It has been known for a number of years that the factors governing vegetative growth are antagonistic to those concerned with reproduction. It was supposed at one period that special internal factors governed the formation and development of the reproductive organs. It remained for Klebs to demonstrate that the phase of reproduction is governed by certain definite external conditions and that reproductive structures do not arise from internal causes alone ; although these no doubt play an important part. Moreover, he was able to show that while the conditions of active vegetative growth are present, reproduction does not occur, and further, that the factors concerned in reproduction are very much more limited than those for growth, so that reproduction is likely to be inhibited by the intensity of some factor being too high or too low.

It must be confessed that experiments on the physiology of reproduction of the higher plants have never been very satisfactory or particularly convincing, and gaps in our knowledge on this aspect of the matter are still very wide. The fungi and algæ, however, have yielded valuable results.

Coons (1916) has studied the relation of growth to reproduction in the fungus *Plenodomus fuscomaculans*, an active parasite of the apple. He discovered that there is only a small amount of growth

in conductivity water, while in distilled water there is better growth and a few pycnidia are formed. Thus, under the experimental conditions employed, conductivity water was the lower limit for growth and distilled water for reproduction. Up to a certain limit, with increase of food, there is an increase in reproduction. Many media suitable at the start are unfavourable at the end. Reproduction is inhibited by a slightly acid medium. With a synthetic medium containing varying amounts of potassium hydrogen phosphate (M/100), magnesium sulphate (M/300), maltose (M/100) and asparagin (M/500), he found that increase or decrease of maltose has a marked effect on reproduction. Light is also essential for reproduction, as well as abundant aeration. The stimulus of light can, however, be replaced by a few drops of hydrogen peroxide, nitric acid, potassium permanganate or ferric chloride, which are all powerful oxidising agents. Coons put forward the view that among the different parts of an organism there is a strong competition for oxygen, and that in conditions which favour vegetative growth, the available oxygen is used for ordinary metabolic processes. If the food supply be reduced, a so-called "hunger-state" sets in, and ordinary respiration is lowered. If the organism be then stimulated by light or some oxidising agent, oxidation of the richer cell contents, such as fats and proteins, sets in, and a large amount of energy is released. "This energy is used for reshaping the reserve foodstuffs into complex protein bodies, the spores."

Barnes (1924, 1925) has shown that a species of *Lachnea* has a conidial stage which falls into the genus *Acmosporium*. The appearance of the sexual or asexual stage is dependent on the nature of the medium employed and also on the amount of light supplied to the cultures.

Light has very definite formative influences upon the fungi. Many will not fruit in the dark, while others will not fruit in the light. Robinson (1926) has shown that the growth and development of the discomycete, *Pyronema confluens*, is conditioned by a number of separate factors which to some extent interact and are dependent upon one another. The effect of the absorption of light energy is only one such factor in the development of the

reproductive structures and apothecia, if and when the mycelium is in a suitable condition for the reception and utilisation of this energy. Although important in themselves, the factors concerned are only one portion of the matter, though they must be favourable, yet a sequence of causation can be recognised. The first stage in the sequence is shown by the definite arrest of development of growth in the main hyphæ of the mycelium, followed by a development of the lateral branch systems, which grow into the air owing to the spacing conditions on the agar surface. Here, moisture relations are altered, and the effect of the energy from the light is shown in morphological changes accompanying the development of the antheridia and oogonia. The pink pigment, characteristic of the fungus, appears at the initial stage of these changes and increases in amount with development. Robinson was unable to ascertain whether the appearance of the pigment and the development of the reproductive structures were causally connected. He considered that if some pigment had not been formed, the dependence of both upon light and the non-appearance of the reproductive structures was certainly significant.

The aerial branches have the potentiality of developing into reproductive structures before they have received energy from light. This is said to explain the appearance of the abortive structures which arise in darkness in equivalent positions to apothecia in normal cultures. The light operates relatively late upon regions of the mycelium when the potentiality for development has already been determined. The absorption of a certain amount of energy from light is therefore a final phase in the sequence of causation concerned in development. Carbon dioxide and excess of carbohydrates inhibit lateral branch structures. Again, humidity relations must be correct before development of oogonia and antheridia takes place. Only a small quantity of nitrate and carbohydrate is necessary for vegetative development, but reproduction only occurs in fluid cultures when the available supplies of nitrogen are becoming exhausted. There is no general development of reproductive structures if the initial concentration of carbohydrate (here maltose) in the medium be higher than $M/250$. Moreover, the moisture content of the air

over the cultures exercises an important effect on the reproductive organs. The most favourable conditions for reproduction are found between relative humidities of 50 and 70 per cent. No antheridia, oogonia or apothecia are formed below 15 or near 100 per cent. relative humidity.

The action of different rays of light on the rate of reproduction of *Volvox aureus* and *Closterium acerosum* has been investigated by Klugh (1925). He found that in blue and red light of the same energy value, reproduction was greater in the case of red light. Green light was quite ineffective in this connection.

Heterothallism.—It was originally shown by Blakeslee (1904) that zygospore formation only occurred in certain members of the Mucorales when hyphae of two different strains come into contact. In the absence of the opposite strain, asexual reproduction continues indefinitely. There are two strains, called respectively plus and minus by Blakeslee, which though morphologically similar are physiologically different, and it is only when the two strains come into contact that the zygospores are produced. Those species in which the presence of two strains is necessary for zygospore formation are termed heterothallic in contradistinction to the so-called homothallic forms, in which no such physiological differentiation exists. Prior to this work, a number of theories had been advanced to account for the somewhat erratic appearance of the sexual organs of the Mucorales. Such factors as respiration, the nature of the medium, water supply, and a number of other causes had all been invoked to explain the production of the zygospores in this group.

Blakeslee and his co-workers (1920, 1921, 1925, 1926, 1927) have now attempted to define further along biochemical lines the differences between the two sexes, with, it must be confessed, little success. According to Satina and Blakeslee (1925, 1926), using the "Gosio Reaction," which involves the capacity of the living cell to absorb and reduce salts of tellurium and selenium to their respective elements, black in the former case and red in the latter, on the average the (+) strains of *Absidia Blakesleana* and *Circinella spinosa* grown on a 2 per cent. agar medium of malt and dextrose and 0.1 per cent. of peptone, show

greater reduction than the corresponding (—) strains. The best results were obtained with either sodium or potassium tellurite (Na_2TeO_3 or K_2TeO_3) rather than with the corresponding selenites, as the red coloured reduction product did not stand out so vividly as the black of the tellurium. With Manolov's reagent (reduction of potassium permanganate), the (+) strains of various species of *Mucor*, *Phycomyces nitens*, *Rhizopus nigricans*, *Syncephalastrum*, *Absidia* and *Parasitella simplex*, again on the average showed a greater power of reduction than the (—) forms. In this respect the (+) strains of the Mucorales behaved like the female sex of the higher animals and dioecious green plants, and the (—) like the male sex. In the genus *Parasitella*, which parasitises other members of the Mucorales, Satina and Blakeslee (1926) have been able to confirm Burgeff (1924) that the parasitism has developed by way of an imperfect sexual reaction. Burgeff, for example, found with species of *Rhizopus* that both the (+) and the (—) forms of *Parasitella* formed galls with either strains of the host. Whereas that which he termed the (+) strain of *Parasitella* only formed galls with the (—) strains of *Absidia glauca* and *A. caerulea* and the (—) race or strain of *Parasitella* with the (+) race of *Absidia*. Satina and Blakeslee were able to confirm the curious cytological phenomena found in connection with gall formation, in which the cell contents of the host and parasite become mingled with the simultaneous formation of the gall. Subsequently outgrowths grow up from the base, which are subtended by a thick-walled storage cell. Unlike Burgeff, they were able to find communication between the gall and the storage cell.

Blakeslee, Cartledge, Welch and Bergner (1927) found that the heterothallic *Mucors* are strictly dimorphic, and that there is no evidence for sex intergrades. Blakeslee and Cartledge (1927) considered that the absence of sex intergrades among heterothallic *Mucors*, is due to the fact that in these forms we are considering gametophytes, whereas in dioecious green plants, in which such intergrades are known, we are considering sporophytes.

Heterothallism has now been found to exist in other members of the *Phycomycetes*. In the *Oomycetes*, Ashby (1922) has shown that *Phytophthora faberi* is heterothallic or dioecious, and Couch

(1926) that species of *Dictyuchus* are also heterothallic. In both these forms, antheridia and oogonia are borne on separate haploid mycelia. Similarly, heterothallism has now been found to exist in the Ascomycetes. Egerton (1914) showed that the presence of two strains (designated by him as (+) and (-)) was necessary for the production of normal perithecia in *Glomerella cingulata*. Since this work a number of publications has appeared, showing that the presence of two strains is necessary for the production of the fructifications in the Ascomycetes. Thus Dodge (1920) showed that antheridia and oogonia appeared along the line of junction of two mycelia of *Ascobolus magnificus*, and similar results were obtained by Betts (1926) for *A. carbonarius*. Derx (1926) has described a heterothallic form of *Penicillium luteum*. Mono-ascospore cultures readily and abundantly produced well-developed ascocarps when they were grown two by two in certain combinations. When this point had been fixed, the different strains isolated from conidia behaved in the same way. ✓ Shear and Dodge (1926) have described heterothallism in the genus *Neurospora*. In *N. tetrasperma*, there are four spores instead of the usual eight in each ascus, and each spore is binucleate. *N. sitophila*, on the other hand, has the usual eight uninucleate spores in each ascus. When grown in culture, *N. tetrasperma* readily produced perithecia, whereas perithecia were only formed in *N. sitophila* at the junction of (+) and (-) mycelia. According to Dodge (1927), and Wilcox (1928), *N. sitophila* presents a true case of heterothallism, and the character distinguishing the (+) from the (-) strain is to be found in the nuclei; this distinction takes place in the second division in the ascus. Gwynne-Vaughan and Williamson (1928) found that single spore cultures of *Humaria granulata* did not develop ascocarps, but that these were formed along the line of junction of (+) and (-) infections. ✓

The Basidiomycetes have been the centre of exhaustive investigations during the last decade on account of this question of heterothallism. Bensaude (1918), in France, and Kniep (1915, 1916, 1917, 1919, 1922, 1923) independently in Germany, showed quite conclusively that the phenomenon of heterothallism exists among the Hymenomycetes. Bensaude, in making cultures of

Coprinus fimitarius, obtained four mycelia each of which was from a single spore culture. Of these four mycelia, only two survived when they were transferred to tubes. The two surviving monospore cultures, which were termed α - and β - by Bensaude, were subcultured, but during eight months they remained in the so-called "primary" condition, *i.e.*, they developed no clamp-connections nor showed paired nuclei and remained sterile. The behaviour of polysporous cultures was quite different. These polysporous cultures were obtained by mixing pieces of the α -mycelium with the β -mycelium, and, as a result, hyphal fusions occurred and a "secondary" mycelium was produced in which the nuclei were paired and divided conjugately, the division of each dicaryon being accompanied by wall formation with a clamp-connection, and fruit bodies made their appearance. Kniep obtained very similar results for *Schizophyllum commune*, but found that fruiting was not necessarily dependent upon the nuclei in the mycelium becoming paired. A haploid mycelium derived from a single spore was able to produce fruit bodies resembling in external appearance and in the production of several ripe spores the fruit bodies of the same species produced on a diploid mycelium, derived from the fusion of two monosporous mycelia. Thus the mere formation of fruit bodies by a monosporous mycelium is no clear indication that the fungus is homothallic. According to Kniep, in a heterothallic species the difference between a haploid fruit body produced on a monosporous culture and a diploid fruit body produced on a polysporous culture lies in the fact that in each basidium of the haploid fruit body there is only one nucleus when the cell is cut off from the parent subhymenial cell, whereas each basidium of a diploid basidium possesses two nuclei. In the haploid basidium the single nucleus divides twice, while in the diploid basidium the two nuclei first fuse and then divide twice.

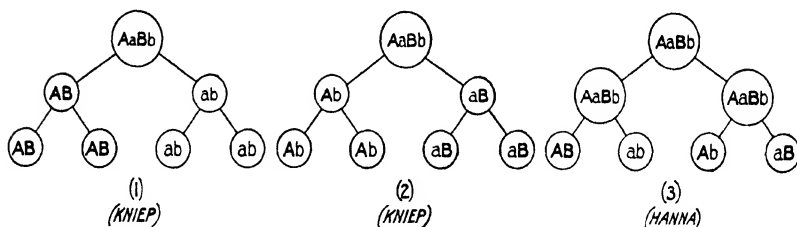
Mounce (1921, 1922) extended these observations of Bensaude and Kniep, and found that both homothallic and heterothallic strains exist in the genus *Coprinus*. Thus *C. sterquilinus* and *C. stercorarius* are homothallic and produce clamp-connections and fruit bodies in monosporous cultures, and *C. lagopus* and *C. niveus* are heterothallic. She was further able to ascertain that the

question of sex in heterothallic Coprini is complicated by the fact that the sexual strains cannot be strictly divided into (+) and (−) groups. For example, in *C. niveus*, she found that in a series of nine cultures, which may be conveniently designated by the letters A to G, A formed clamp-connections with D, D with C, F with E, and B formed clamp-connections with F, H and G, yet B and D did not form clamp-connections.

According to Kniep (1922, 1923), *Schizophyllum commune* and *Aleurodiscus polygonius* possess four sexually different kinds of spores. Kniep assumed that in these strains sex is determined by two allelomorphic pairs of factors which are present in the fusion nucleus of the basidium, and that these become segregated in the two subsequent nuclear divisions according to Mendelian principles. If these pairs of factors are represented by the letters (Aa) and (Bb), then the fusion nucleus of the basidium will have the composition (AaBb). At the second division of the fusion nucleus, the sex factors will be separated as (AB), (ab), (Ab) and (aB), and four different kinds of spores will be formed. Only those spores without a common factor will unite sexually in the mycelial stage. Thus (AB) will combine with (ab), whereas (AB) and (aB) will not unite since they both carry the common factor (B). Kniep was able to show that in *Aleurodiscus polygonius* each basidium bears two pairs of spores, one of each sex. He also ascertained that the basidia of a single sexual strain were of two kinds, one kind bearing the spores : (AB), (AB), (ab) and (ab), and the other (Ab), (Ab), (aB) and (aB). He concluded from these results that the reduction of the chromosomes takes place in the first division of the fusion nucleus and not in the second.

In an extensive investigation of *Coprinus lagopus*, Hanna (1925) found that the spores from any individual fruit body belonged to four sexually different groups. The basidial analyses showed, however, that though some of the basidia bore spores of two sexes only, a pair of one sex and a pair of another and opposite sex, other basidia bore spores of all four sexes : (AB), (ab), (Ab) and (aB). Hanna concluded from the presence of four sexually different kinds of spores on a single basidium in *C. lagopus*, that the reduction process was brought about in the second, and not

at the first division of the fusion nucleus. The conclusions of Kniep and Hanna are shown diagrammatically below :—



Funke (1924) came to a similar conclusion to that of Hanna for *Hypopholoma fasciculare*, *H. capnoides* and *Collybia velutipes*, namely, that the reduction took place in the second and not in the first division of the fusion nucleus. Vandendries (1924) found that each strain of *Coprinus radians* sexually has two different kinds of spores. All possible crossings were made between twenty-three monosporous mycelia derived from a single fruit body, and secondly between twenty-five monosporous mycelia derived from another fruit body. Without exception, in the first experiment, the spores proved to be of two sexes. In the second experiment, twenty-four of the twenty-five proved to be of two sexes, while the twenty-fifth behaved in an anomalous manner and reacted with all the other spores. Vandendries found that twenty-seven of his cultures changed spontaneously from the haploid to the diploid condition. He advanced (1925) the theory that all species of *Coprinus* are at first heterothallic. In other words, the spores of the homothallic, as well as the heterothallic species are at the commencement unisexual, and he suggested that in the homothallic species, such as *C. sterquilinus*, the mycelia change at a very early stage in their development from the haploid to the diploid condition, but in the heterothallic species development is delayed over a longer period which may be of several weeks or even months. Vandendries therefore considered that *C. sterquilinus* is not homothallic, and that *C. radians* is not heterothallic, but considered that they both show a condition to which he has given the name "heterohomothallic." Brunswik (1924) showed

that the sexual reactions of *Coprinus curtus*, *C. deliquescens*, *C. ephemerus* and *C. velaris* are similar in their results to *C. radians*, but explained his observations by the presence or absence of sterility factors rather than by sex factors.

Newton (1926) discovered that *Coprinus Rostrupianus* was heterothallic. All monosporous cultures during the first few weeks were entirely unisexual, half being of one sex, and half of the opposite sex. Of twenty-five monosporous cultures which were kept under continuous cultivation for six months, eleven remained haploid and fourteen passed spontaneously into the diploid condition. *C. Rostrupianus*, unlike *C. lagopus*, is bisexual, and the basidia always bear two kinds of spores, two of one sex and two of the opposite sex. Here sex is apparently concerned with one pair of Mendelian factors (Aa). Newton found that in certain cases the segregation of sex factors takes place in the second division for *C. lagopus* and *C. Rostrupianus*. The method of proving this fact was ingenious in the extreme. If segregation of the sex factors involved in *C. Rostrupianus*, (A) and (a) take place in the first division, then the two spores bearing (A) should have adjacent positions in every spore tetrad. If, however, segregation take place in the second division, the two spores (A) should occupy adjacent positions in some basidia and diagonal positions in others. In an analysis of six basidia, the two spores had adjacent positions in four basidia and diagonal positions in two. In *C. lagopus* there are apparently three types of basidia, a four-sex type : (AB), (ab), (Ab), (aB) ; a two-sex type : (AB), (AB), (ab), (ab) ; and a further sex-type : (Ab), (Ab), (aB), (aB). Of the three types of basidia in the hymenium of any fruit body, 50 per cent. are of the first type, and 25 per cent. of each of the other two types. Newton considered that the results here are best explained on two assumptions : (a) that the two sex factors in the nucleus of each spore are carried by two separate chromosomes, one factor on each ; and (b) that in some basidia the segregation of both sex factors takes place in the first division of the fusion nucleus of the basidium and in the other basidia, the segregation of one pair takes place in the first division and of the other pair in the second division.

Coprinus lagopus does not pass spontaneously from the haploid to the diploid condition (Hanna, 1928).

Heterothallism has now been recorded in the Uredinales. Craigie (1927) found that in *Puccinia Helianthi*, when the basidiospores were shed on the leaf of the sunflower, the spermatogonia made their appearance in about eight days. In ten or eleven days after sowing, when the mycelia from two different infections overlapped, æcidia were discovered in 50 per cent. of cases. The rest of the infections, either simple or compound, did not show any signs of æcidia for three weeks. Later, however, approximately 50 per cent. did so. It is clear from these results, that the presence of two mycelia, evidently of two different strains, stimulated the production of the spore fruits. Craigie was also able to show that when the contents of several spermatogonia were mixed and added to the infections, in nearly every case æcidia were formed. Craigie regarded the two heterothallic strains as being of different sexes. Similar results were obtained for *P. graminis*.

Kniep (1926) and Dickinson (1927) have discovered heterothallism existing in the Ustilaginales. In *Ustilago antherarum*, as well as in other species, two or possibly more strains are present, and fusions take place between cells of the opposite strains. The best external conditions for the occurrence of cell fusions were found to be abundance of oxygen or lack of appropriate food material.

Dame Helen Gwynne-Vaughan (1928), in her presidential address to Section K of the British Association, has advanced an explanation based on nutrition to account for heterothallism. In the Ascomycetes, for example, *Humaria granulata*, which lacks an antheridium, and in which the ascocarp is developed from an oogonium, two strains are necessary before the fruit body makes its appearance. If the (+) mycelium be assumed to be a saltant, which, as an hereditary character, possesses the power of extracting some essential food substance (A) from the substratum for the formation of the ascocarps, but does not possess the power of absorbing some other equally essential substance (B), and, further, if the assumption be made that the (−) strain can extract (B)

rapidly but not (A), then when the two strains meet, the two essential conditions for ascocarp formation are present, and ascocarps are produced. Similarly, in the Hymenomycetes, certain forms such as *Aleurodiscus polygonius* and *Coprinus lagopus* are described as quadrisexual. Gwynne-Vaughan considered it difficult to imagine a race with four sexes, but a race requiring four or more food substances in preparation for the fruiting period is more probable. Thus, if of the four characters termed (A), (a), (B) and (b) which these fungi have been shown to inherit on Mendelian lines, each represents the power of rapidly withdrawing from the substrate some essential food substance, and again, if each spore contain either (A) or (a) and either (B) or (b), then the necessary conditions for sporophore formation will be assumed when (AB) and (ab) or (Ab) and (aB) meet.

The Nature of Growth Curves

A number of attempts have been made within recent years to give mathematical expression to the growth curves of plants. It was shown many years ago by Sachs, that in general terms the growth of a plant could be expressed as an S-shaped curve. The growth rate began slowly, and was followed by a rapid period of increase, the so-called "grand-period of growth," and this period of rapid increase gradually fell away.

Fungal hyphæ grow rapidly at first, and then fall into a rate dependent on the medium, temperature, and the nature of the fungus. The extension in growth of fungal hyphæ occurs exclusively at the tip. Henderson Smith (1924) found that if the distance were measured between the tip and some recognisable point on a hypha of *Botrytis cinerea*, the rate of growth was at first slow and gradually increased to a maximum, at which it remained constant for a time; branch hyphæ showed a similar increased rate of elongation. If the whole hyphal system (parent and branches) were taken as a unit, then the rate of growth of the whole system remained constant for many hours. This latter result was said to be due to the difficulty of passing food forward quickly enough to the actively growing hyphal tip. The concen-

tration of the food was no longer the same, and a pressure gradient was established from the tip backwards. The tip, therefore, though it increased in length, no longer advanced at a rate proportionate to the whole length of the hypha, but fell more and more behind that rate.

In the growth of yeast there is first a lag-phase, which consists of a quiescent period of one to two hours varying with the individual cells, and then growth takes place at an unrestricted rate for a time and this in turn falls away. According to Slater (1918), measurements of the lag-phase in yeast show that old cells remain quiescent for a time when introduced into fresh medium. They then start growing rapidly at the normal unrestricted rate. On the other hand, little or no lag is shown in yeast cells grown from spores when once development has started. The growth of the yeast proceeds in alternate periods of rest and growth, and the observed lag is apparently little more than a prolonged period of rest.

If yeast be seeded into malt wort, the following stages of growth may be observed : (a) a lag-phase or period of quiescence ; (b) a logarithmic phase or period of unrestricted growth ; (c) retardation in growth due to carbon dioxide ; (d) retardation due to failure of oxygen supply. It is this last factor which finally brings growth to a standstill. It is on the factors of seeding, aeration, temperature, etc., that the different stages of growth become prominent, overlap or disappear. If the seeding consist of actively growing yeast, the lag-phase disappears, if the seeding amount to a few million per cubic centimetre, retardation of growth at once sets in, and there is no logarithmic period ; if the seeding be very great, lag in growth and rapid accumulation of retarding influences may prevent growth entirely.

Gregory (1921) found that the increase in length, breadth and area of the leaves of *Cucumis sativus* show a grand period of growth under normal conditions in full sunlight. Three sets of experiments were undertaken, the seeds being sown in November, February and June, respectively. Each experiment lasted for thirty days. The type of curve found for the growth of the leaves in November was of the typical S-shape, which is found for auto-

catalytic reactions ; whereas, in March and June, the curves followed the exponential curves of the general type given by the equation :—

$$A = ae^{rt}$$

where A is the total area after a time t and a and r are constants. Such an expression may be written as :—

$$A = ax^t$$

where $x = \log_e r$, from which it will be seen that the growth

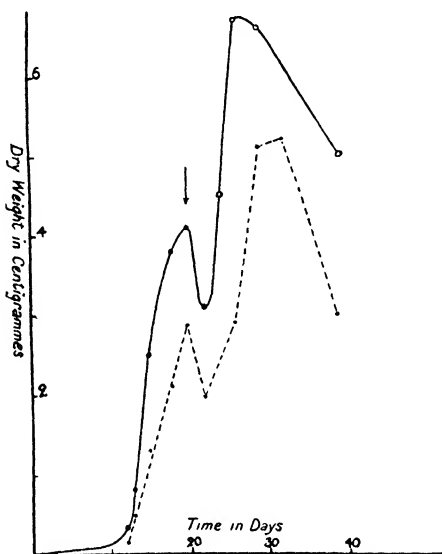


FIG. 51.—The dry-weight ratio for roots from cuttings of tomato (Princess of Wales). The arrow indicates the time of appearance of secondary roots. (After Priestley and Evershed, *Annals Bot.*)

followed a compound interest law. In December, however, the increase in leaf area followed a more complex law, which was expressed by the equation :—

$$A = at^r$$

where A , e , r and t had the same values as before, and the actual rate of increase at any time could be ascertained by the formula :—

$$\frac{dA}{dt} = \frac{rA}{t}$$

showing that the rate of increase was still proportional to the leaf area extant, but tended to fall away with time, owing to the presence of some detrimental factor. When the plants were grown in continuous artificial light, the rate of increase fell away with the first measurement in area. Gregory supposed that this detrimental factor might well be the high temperature, which had to be maintained during the course of the experiments conducted under conditions of artificial light, which led to an increase in the rate of respiration. Gregory (1914) has also found that the "average leaf area" was determined by the product and the intensity of the light.

Priestley and Evershed (1922) have quantitatively analysed the conditions necessary for the formation of roots from cuttings of *Tradescantia zebrina* and the tomato, based on calculations with wet and dry-weight material. The curves obtained were a sequence of S-shaped curves (Fig. 51). The time of transition from one curve to the next coincided with the time of appearance of a new crop of roots of a secondary order. In an interpretation of these results, Priestley and Pearsall (1922) looked upon the logarithmic phase of increase as the natural phase of increase, where increase in mass is an exponential function of the time; the phase of retardation, in which the growth rate is directly proportional to time, is said to be due to the operation of inhibiting factors, such as the accumulation of the end-products of metabolism, which have been left over from the previous unrestricted growth.

Using the large mass of data gathered for the growth of maize by Kreusler and his co-workers, Briggs, Kidd and West (1920) have analysed the growth of this plant in terms of dry-weight, leaf area, and time, and have also employed the measure: "relative growth rate," which they defined as the weekly percentage increase in dry-weight plotted against time and the "leaf area ratio" which was the leaf area increase in square centimetres plotted against time.

It was found that the growth rate of maize varied at different times of its life in a perfectly definite manner. It was discovered that in the first stage there was an actual decrease in weight,

evidently to be correlated with losses due to respiration. Then followed a phase of rapid increase rising to a maximum, which passed into a steady and continuous fall. Subsidiary maxima made their appearance in the descending part of the curve which coincided with the appearance of the male and female inflorescences. It is possible that these secondary maxima which made their appearance were due to strong secondary reactions, for there was a marked rise in respiration at such a time. Of the various environmental factors, temperature is said to have more bearing than light.

In a second paper of this series, Briggs, Kidd and West (1920) have expressed the rate of growth per unit of leaf area instead of unit dry-weight, and have made use of a third relation: "unit-leaf rate," which is defined as the "weekly rate of increase of dry-weight in milligrammes per square centimetre." They found that the unit leaf rate did not undergo a perfectly definite type of variation as did the relative growth rate, but fluctuated about a mean value. It was this unit leaf rate which was so closely correlated with temperature rather than with any other external factor.

The growth curves of the cotton plant have been analysed for the conditions prevailing in India by Inamdar, Singh and Pande (1925). The growth curve showed a maximum increase which was reached sooner or later depending on the vegetative phase. The shorter the vegetative phase, the earlier was the maximum reached. No grand period of growth was discovered. Initial comparison of the growth rate curves with the leaf area ratio and leaf weight ratio showed no agreement between them. This was possibly due to the fact that the leaves had not yet reached their maximum assimilating capacity. There was an active phase of growth when the growth rate curve ran parallel to the curve of either the leaf weight ratio or the leaf area ratio curves. It was further ascertained that there was a greater decrease in the growth rate curve in the later stages of the life-cycle of the plant than could be accounted for in the percentage leaf weight ratio or leaf area ratio.

V. H. Blackman (1919) considered that the growth of an

annual plant follows a compound interest law. He pointed out that in many natural phenomena, processes are to be found in which the rate of change is proportional to the quantity itself. Money placed at compound interest accumulates in this way, and the rate at which a body cools follows a compound interest law. Blackman went on to show that assimilation and growth are closely correlated : " If the rate of assimilation per unit area of leaf surface and the rate of respiration remain constant, and the size of the leaf system bears a constant relation to the dry-weight of the whole plant, then the rate of production of new material, as measured by dry-weight, will be proportional to the size of the plant, *i.e.*, the plant in its increase in dry-weight will follow a compound interest law."

In any annual plant the ultimate dry-weight will depend (1) on the weight of the seed ; (2) on the rate at which the material present is employed to produce new material, *i.e.*, percentage increase in dry-weight per day or week or other period ; and (3) the time during which the plant increases in weight.

Gressler (1907), working with *Helianthus annuus* treated the matter as though it were a discontinuous geometric series. Blackman held it to be continuous, a very much more probable assumption, and substituted the equation :—

$$W_1 = W_0 e^{rt}$$

to express the growth of an annual plant rather than Gressler's equation :—

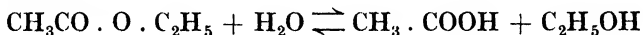
$$W_1 = W_0(1 + r)^t$$

where W_1 is the final weight, W_0 the initial weight, r the average rate of interest, t the time, and e the base of natural logarithms. Blackman considered that r is an important physiological constant. It represents the efficiency of the plant as a producer of new material, and he termed r the " efficiency index " of the plant. From a consideration of this conception two factors are necessary for the best production of vegetative material by an annual plant : (1) a large seed, and (2) a high economy of working represented by a large efficiency index. It is clear that a good start

means a larger capital to work on, for the efficiency of the plant is highest in its earliest stages of growth.

Kidd, West and Briggs (1920) considered that a rigid application of the compound interest law throughout the life of the plant is impossible, and that r is not a constant at all. But as Blackman (1920) has himself stated, these workers are under a misapprehension regarding the nature of r . The value r must be taken as an average, not as a rigid constant in the same sense as a physical constant.

Blackman's views on growth are not universally held, and a second group of workers, especially prominent in America, consider growth to be of the nature of an autocatalytic reaction. As the name implies, an autocatalytic reaction, is a reaction capable of self-catalysis, one of the products of the reaction acting as a catalytic reagent. An example of an autocatalytic reaction may be found in the hydrolysis of esters by water. The monomolecular reaction between ethyl acetate and water to give acetic acid and ethyl alcohol :—



is one such case. The reaction at first proceeds slowly, and gathers velocity with increase in the concentration of the acetic acid split off in the course of hydrolysis. The acetic acid here acts as the catalytic reagent, for the presence of acids markedly favours hydrolysis of esters. The curve for such a reaction is typically S-shaped.

From the similarity in the shape of the curves, Robertson (1908) advanced the view that growth is of the nature of a monomolecular autocatalytic reaction and that some special catalyst of the nature of an enzyme governs the growth rate of an organism. Robertson, from his experimental results, supposed that in any particular growth cycle, either of the organism as a whole or of any part of the organism, the greatest increase of volume or weight in any unit of time takes place when the total growth due to the cycle is half accomplished. Such a growth-cycle is said to conform to the equation :—

$$\log \frac{x}{A-x} = K(t-t_1)$$

where x is the amount of growth in weight or volume which has occurred in the time t , A is the total amount of growth attained during the cycle, K is a constant, and t_1 is the time in which the growth-cycle is half completed.

Certainly Robertson's figures expressed in the form of a curve show an excellent agreement with such an expression, although, when expressed as a percentage, they are too large to support his views.

If growth were simply due to the result of an autocatalytic reaction, once it had attained its maximum point, a constant value should be recorded. This is not the case in practice, and, with advance in time, a loss is found. This is considered due to secondary changes.

Reed and Holland (1919) strongly supported Robertson in this respect. They found that the growth rate of the annual *Helianthus annuus* approximated to that of an autocatalytic reaction, in which the growth rate at any particular time could be expressed by the equation :—

$$\frac{dx}{dt} = Kx(a-x)$$

where a is the initial quantity of material subject to transformation, x is the amount transformed at a time t , and K is a constant. According to these investigators, the growth rate is governed by constant internal factors rather than external factors.

Again Reed (1920) found that the growth rate of apricot shoots followed a definite but fluctuating rate. The maximum rate was shown soon after the season's growth had begun ; but at the same time three intra-seasonal growth-cycles were exhibited. The growth rate in each cycle closely followed the rate of an autocatalytic reaction. The growth rate for the entire season conformed to that of a reaction consisting of two monomolecular reactions, one of which at first accelerated and later retarded the other. Reed measured the rate of increase in height of walnut trees. The young trees showed distinct cycles of growth in a single season, but in each cycle the rate of growth conformed to an autocatalytic reaction. Reed (1921) has emphasised the fact that

a relation exists between the rate of growth and the final size of the plant. The amount of growth yet to be made appears to be an essential function of the final size. The rate may be affected by two factors: (1) a variation in the supply of catalysts; and (2) a variation in the supply of potential growth material.

The "summation curve" (the total number of flowers up to a given date) in the case of the Egyptian cotton plant has been found by Prescott (1922) to be typically S-shaped and can be expressed by the equation:—

$$\log \frac{x}{a-x} = K(t-t_1)$$

where a is the total number of flowers obtained, x is the number of flowers up to a given time t , t_1 is the time when $x = \frac{a}{2}$, and K is a constant. In other words, the summation curve appears to follow an autocatalytic reaction.

Mitscherlich (1919) has evolved the equation:—

$$\log ({}^n\sqrt{A} - {}^n\sqrt{y}) = \log ({}^n\sqrt{A} - cx)$$

to express the growth of a plant, in which n is a variable quantity indicating the probable number of external factors, A is the maximum possible dry-weight attainable by the plant in question, y is the dry-weight of the plant at a time x (x being expressed in vegetation periods of any arbitrary length) and c is a constant. It is doubtful if such an expression has any significance in practice. An equation of this kind may fit the facts very well in one case, yet be found to be wanting in another. Rippel (1919) has strongly criticised such an expression and considered that Robertson's equation gives a more nearly correct representation of physiological processes than that of Mitscherlich.

West, Kidd and Briggs (1920), Vyvian (1924), and Briggs (1928) considered that the S-shaped curves of monomolecular reactions and the S-shaped curve obtained for the growth of a plant show but a superficial resemblance which is of no fundamental importance, and they held that the falling off in the growth rate per unit dry-weight was due to increasing differentiation into productive and non-productive tissue. Gregory (1928n)

has replied in detail to these criticisms, and the originals should be consulted.

An important criticism has been brought forward by Snell (1929) against the autocatalytic theory of growth. He considered that the equations which these various workers have used cannot apply in the case of living organisms, because of the effect of increasing size on the concentration of the reagents involved in growth. The equations, based on the assumption that growth is of the nature of an autocatalytic reaction, hold true only on the condition that the volume occupied by the reacting substances remains constant, and since a growing organism is constantly increasing in volume this condition is not met. When a chemical reaction is carried out in the laboratory, the reagents are ordinarily dissolved in water or some solvent, and the volume of the solvent is kept constant throughout the reaction, and it is to such reactions carried out under such conditions that the usual formulæ based on the law of mass action are intended to be applied. Snell therefore considered that the equations for growth should include a volume term. Thus if A represents the concentration of the substrate, x the amount of the end-product at a time t , V the volume of the organism at the beginning of the growth-cycle, then the corrected equation becomes :—

$$\frac{dx}{dt} = K_1Ax - K_2 \frac{x^2}{cx + V}$$

where c is a constant. Such an equation gives a sigmoid curve, but one of decidedly different form to that of Robertson, and, in fact, it is very doubtful if it can be applied to the majority of growth curves.

Richards (1928) found that the growth curve of yeast was an asymmetrical S-shaped curve. Increase in growth in this particular case was measured as the increase in the number of cells in a unit volume. Robertson's expression only applies to asymmetrical S-shaped curves, and, since the curve here is asymmetrical, it must be concluded that yeast growth is not limited by a monomolecular autocatalytic reaction. From a consideration of the equations of multimolecular reactions, and making the assumption of a slowest

limiting master reaction, Richards concluded that this master reaction for yeast was of five molecular complexity.

The average plant in its natural surroundings is exposed to a large and ever-changing variety of external factors, such as variations in temperature, rainfall, light intensity, etc. Yet, in spite of these continually changing conditions, it manifests a steady rate of growth till it reaches maturity. The plant is a complex physico-chemical system composed of co-ordinated chemical reactions and these co-ordinated reactions no doubt conform to Le Chatelier's theorem, which states: "When one or more of the factors determining an equilibrium is altered, the equilibrium becomes displaced in such a way as to neutralise as far as possible the effect of the change." Action and reaction are continually taking place in the plant, and every change in the surrounding environment must be met by a corresponding response in the plant.

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